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EDITORIAL

Polyunsaturated Fatty Acids Are Relevant – From New Mechanisms of Action to Healthy Benefits in People

Is it possible that the quality of food plays a role in the obesity epidemic that the world is experiencing? Pediatric obesity in children has taken on epidemic proportions, and a recent study has explored if associations between dietary polyunsaturated fatty acids and adiposity exist in children.

Is there an interaction between aspirin use and taking prescription omega-3 fatty acids in the incidence of gastro-intestinal lesions, a common side effect of non-steroidal anti-inflammatory drugs? This is an important topic given that aspirin is taken by so many people around the world.

Pharmacological approaches that aim to reduce LDL levels remain an important topic of research and development, since cardiovascular disease is still the major cause of death in many parts of the world. *But how do omega-3 LCPUFA affect the prominent new drug target PCSK9?*

What are the contributions of alpha-linolenic acid and omega-3 LCPUFA to the benefits of a healthy diet? A recent study that assessed the effect of meeting or not meeting the internally-recommended intake levels of these fatty acids has provided some remarkable insight into one's risk of disease and death.

A fairly high percentage of pregnancies is complicated by gestational diabetes, with serious health risks for both mother and child. But researchers now think something is going seriously wrong with the provision of DHA to the fetus in this condition. *Can something be done about this?*

These five questions are important because they involve large numbers of people worldwide. In this issue of Fats of Life, we will discuss some recent representative studies on these topics, from among the wealth of ongoing research suggesting that polyunsaturated fatty acids are very relevant to human health.

While the nutritional and clinical science around PUFA for human health progresses, basic biochemical and cell biological research is also moving ahead. To give you a taste of some fundamental research findings, we highlight two elegant studies. A specific phospholipase enzyme has been identified to couple the selective release of EPA and DHA to the formation of anti-inflammatory and tissue protective lipid mediators. Both the human and mouse enzyme appear to share this activity. The second study has identified a DHA-derived lipid mediator that regulates the development of human B-lymphocytes into a differentiated cell-type that produces immunoglobulin E, the key mediator of allergic reactions. It suppresses the formation of IgE-cells through a unique mechanism, a finding that will likely inform future studies to better appreciate how the development of allergies and inflammatory reactions may be controlled.

This issue's Guest Article is contributed by professor Richard Bazinet. At the Department of Nutritional Sciences at the University of Toronto, Canada, he studies the mechanisms that regulate brain lipid metabolism and signaling, with the aim to better understand the role of brain lipid metabolism in the pathogenesis of neurodegenerative diseases and neuropsychiatric disorders. His article will provide you with an interesting overview and current interpretation of how the central nervous system obtains the DHA it needs.

We hope that you enjoy this April newsletter.

Gerard Bannenberg, Ph.D.
Editor

CARDIOVASCULAR HEALTH

Adequate Dietary Intake of Omega-3 LCPUFA by People Aged 55-80 Years is Associated with a Markedly Reduced Risk of Death from Cardiovascular and Coronary Heart Disease in the Context of a Mediterranean Diet

THIS ARTICLE AT A GLANCE

- *This study has examined the relationship between ALA and omega-3 LCPUFA intake, and the risk of developing cardiovascular disease and death in older adults.*
- *People who were at known risk of developing cardiovascular disease and who met international recommended intake levels of ALA (≥0.7 en%) and omega-3 LCPUFA (≥500 mg/d EPA plus DHA) in the context of switching to a Mediterranean diet had a 37% reduced risk of dying from any cause.*
- *The study showed that meeting omega-3 LCPUFA intake recommendations was associated with a reduction in coronary heart disease and sudden cardiac death by ~50%.*
- *Consumption of extra-virgin olive oil by people meeting ALA intake recommendations was associated with additional reduced total mortality.*

People adhering to a Mediterranean diet (or Mediterranean-style diet) have reduced risks of cardiovascular and metabolic disease, as well as lower all-cause mortality, when compared to most people in Westernized countries with other dietary habits. The Mediterranean diet is a dietary pattern, with some variation among the Mediterranean regions, but with a number of common elements: the use of olive oil as the main culinary fat, a relatively high consumption of fruit, vegetables, legumes, and unprocessed cereals, water with meals, frequent but moderate consumption of wine, fish and seafood, nuts, sauces made with onions, garlic and leeks, dairy (cheese and yoghurt), and poultry intake, and a relatively low intake of red meat and seed oils. An important nutritional aspect of the traditional Mediterranean diet is a comparatively high intake of omega-3 polyunsaturated fatty acids (omega-3 PUFA), from alpha-linolenic acid (ALA) to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Mediterranean diet is also regarded as a cultural heritage with an important social component.

A compelling body of epidemiological studies supports the concept that adherence to a Mediterranean diet results in a lower incidence of cardiovascular disorders and disorders related to metabolic health, such as type 2 diabetes. Short-term effects of the Mediterranean diet relate to improvements in blood lipid profiles, and anti-inflammatory effects are noted after longer intake durations. An important prospectively controlled intervention study that has highlighted the cardiometabolic benefits of the Mediterranean diet is the PREDIMED study, carried out from 2003 to 2011. Follow-up studies continue to be performed in order to learn as much as possible from this unique primary prevention trial.

The PREDIMED trial was a multicenter, parallel group, randomized clinical trial focused primarily on determining the effects of supplemental intake of extra-virgin olive oil and nuts in the context of the Mediterranean diet on the primary prevention against cardiovascular disease in a high-risk cohort. This cohort consisted of people 55-80 years of age, with no history of cardiovascular disease at study onset, but who had a relatively high cardiovascular risk - participants had either type 2 diabetes or at least three of the following car-

People adhering to a Mediterranean-type diet demonstrate reduced risks of cardiovascular and metabolic disease, as well as lower all-cause mortality, in comparison to most people in Westernized countries with other dietary habits.

diovascular risk factors: overweight or obesity, hypertension, dyslipidemia, current smoking and a family history of early onset coronary heart disease. The majority (about 92%) of participants did not habitually consume a complete Mediterranean diet at baseline. Participants had been recruited through 11 field centers, each reaching some 20 physician practices on average. The eligible participants (7447) were allocated randomly to one of three groups: *i)* a Mediterranean diet with supplemental extra-virgin olive oil, *ii)* a Mediterranean diet with supplemental nuts (walnuts, almonds and hazelnuts), and *iii)* a control diet consisting of advice to reduce all types of dietary fat. At baseline and during yearly follow-up a dietary assessment was made with a validated food-frequency questionnaire. PREDIMED trained and certified dietitians and nurses continuously helped participants maintain their assigned diets throughout the seven-year study, resulting in relatively high study adherence.

In 2013, the results of the study were published. In both Mediterranean-diet groups the incidence of cardiovascular disease, as a pooled outcome of myocardial infarction, stroke and mortality from cardiovascular causes, was markedly reduced compared to the control group (around 30% reduction in event incidence). All-cause mortality decreased in the extra-virgin olive oil group but was indistinguishable in the control and supplemental nuts groups. The risk for stroke, but not death from cardiovascular causes, was significantly reduced in both Mediterranean diet intervention groups merged together compared to control subjects (with a low fat diet). The PREDIMED study made a very important contribution to the recognition that a change in dietary habits towards the consumption of a limited number of specific food items in the context of adherence to a Mediterranean diet can make an enormous difference in the probability of actually developing cardiovascular disease in people that are at known risk, a situation that is very common in the Westernized world. According to projections published in the 2016 Heart Disease and Statistics update of the American Heart Association, over 70% of American men and women will most probably still remain with a poor diet score by 2020.

In a follow-up of the PREDIMED study, Sala-Vila and colleagues assessed if ALA and omega-3 LCPUFA intake were associated with the development of cardiovascular disease in PREDIMED participants. More specifically, they investigated whether adherence to recommended daily dietary intakes of ALA (0.7% of total energy) and to total omega-3 LCPUFA (500 mg/d of EPA plus DHA), as defined by ISSFAL, is related to the development of the risk of all-cause mortality and death from cardiovascular disorders. Although the dietary advice in the PREDIMED study achieved sustained differences in the intake of nuts and extra-virgin olive oil, the dietary intake of many other dietary components did not differ greatly between the three groups during the trial, including fish/seafood and legumes. This allowed the analysis of the relationship between omega-3 PUFA intake and the incidence of death from cardiovascular and other causes by all cohort participants.

The dietary intake of omega-3 PUFA was obtained from records of food frequency questionnaires in combination with Spanish food composition tables, allowing the calculation of individual dietary intake of omega-3 LCPUFA intake (from seafood/fish) and of ALA (from soybean oil, walnuts, margarine, corn oil, sunflower oil and olive oil, with the latter three oils containing negligible levels of ALA). The validated questionnaire had also been substantiated against three-day food records. Hazard rates of all-cause mortality, fatal cardiovascular disease, fatal coronary heart disease (acute myocardial infarction, unstable angina, other forms of chronic ischaemic heart disease), sudden cardiac death, and fatal stroke, were determined for the two exposures over a 5.9-year (mean) follow-up time interval. Hazard ratios were used to express relative longitudinal event risks. The associations between omega-3 PUFA intake and hazard rates could be determined for a total of 7202 study participants (data from people with incomplete dietary assessments, implausible fatty acid intakes, or exceedingly low or high total energy intake were excluded).

At baseline, 76% of the entire study cohort did meet the daily intake recommendation for omega-3 LCPUFA, but only 22% met the recommended intake of ALA. This suggests that a major proportion of PREDIMED trial participants, while at predicted risk for cardiovascular disease, had a relatively high background of fish consumption (typical for Spain), and a relatively low intake of ALA from vegetable origin. The study reports that while participants shifted to a Mediterranean diet, those individuals that furthermore had an omega-3 LCPUFA in-

Analysis of the PREDIMED study found that meeting international recommended intake levels for ALA (0.7% of total energy) and EPA/DHA (500 mg/d) is associated with markedly reduced cardiovascular disease incidence and death from all causes in older people at risk for developing cardiovascular disease and who switched to a Mediterranean diet.

take above 500 mg/d displayed markedly reduced risks of dying from cardiovascular disease (39% lower, P-value 0.032), coronary heart disease (46% lower, P-value 0.046), or sudden cardiac death (51% lower, P-value 0.069). No significant associations between omega-3 LCPUFA intake and all-cause mortality or fatal stroke were found. Interestingly, a dietary intake of ALA above 0.7% energy correlated with a markedly reduced risk of death from all causes (28% lower) compared to participants below this level of ALA intake. No specific associations between ALA intake and death from cardiac causes or stroke were identified.

When the hazard rate was determined for participants that met both ISSFAL recommendations for adequacy in daily ALA and omega-3 LCPUFA intake (1296 people), and was compared to that of participants meeting neither recommendation (1431 people), a 37% reduction in longitudinal risk of death from any cause was identified. This result suggests that a change to a Mediterranean diet in older people (average age 67 years) at known risk for cardiovascular disease and achieving an intake of omega-3 LCPUFA and ALA above internationally recommended levels, is associated with a substantial reduction in the chance of dying in the ensuing 6-7 years. The researchers next made a separate analysis of the effect sizes of the associations of ALA adequacy with total mortality in participants assigned to each of the three original treatment arms. Interestingly, those individuals that originally received the supplemental extra-virgin olive oil displayed the strongest reduction in total mortality (49% reduction in hazard rate compared to the control group).

A major achievement of the PREDIMED study was to keep attrition levels low. The free provision of virgin olive oil and nuts, and close follow-up of the intervention groups by certified dietitians and nurses, was designed to help maintain study compliance. The large size of the study was also helpful to identify specific associations of omega-3 PUFA intake with substantial reductions in risk. A limitation of this study is that the results are generalizable only to older people with a risk for cardiovascular disease and who switch to a better diet; however, this age group is likely of high relevance since susceptibility to chronic disease increases at this age. The results are therefore of great relevance, indicating that a Mediterranean-type diet with a sufficient daily intake of omega-3 LCPUFA plus ALA may lead to a substantially reduced risk of cardiovascular disorders and death from cardiovascular and non-cardiovascular origin in this age and risk group. In those participants with an adequate ALA intake, the supplemental intake of nuts, and extra-virgin olive oil in particular, was associated with further beneficial effects on health. Olive oils contain a range of secondary plant metabolites that have reported positive health benefits, with higher concentrations being present in less refined extra-virgin olive oils.

As with any observational study, the measured associations cannot be formally used to conclude that the observed relationships are causal, since unidentified confounding factors may explain or contribute to the decreases in disease incidence and mortality. The statistical models were adjusted for a range of potential confounder variables such as age, sex, BMI, smoking status, physical activity, total energy intake, history of diabetes, hyperlipidemia, hypertension, alcohol intake, and dietary factors (fiber, vegetables, fruits and red meat). Small differences in many of these factors were observed in cohort sub-groups that met or did not meet the recommendations for ALA and omega-3 LCPUFA intake. One example of a potential confounder that was not corrected for is linoleic acid intake, a known determinant for the kinetics of omega-3 PUFA tissue and membrane distribution and metabolic interconversion of ALA to longer-chain omega-3 PUFA.

The results of this study are in support of recommended daily intake levels for ALA and EPA/DHA to offer protection against the development of cardiovascular disease and contribute to health. Additional bioactive components found in our diet are indicated to provide further health benefits.

Whether the conclusions of this interesting study hold for other age groups and distinct populations for primary and secondary prevention will be the subject of further controlled intervention studies. The results of PREDIMED have been instructive to public health strategies to improve citizens' eating behavior, and further support the Mediterranean diet as especially healthy. These results support the body of knowl-

edge that both long-chain omega-3 PUFA and ALA make important contributions to cardiovascular and general health. Understanding which components of healthy diets are important for people will also gradually improve recommendations in the context of human behavioral habits that are unhealthy, for example a high prevalence of a sedentary life-style.

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FOL

Evaluating PCSK9 as a New Point of Control for the Actions of Omega-3 LCPUFA on Blood Lipids

THIS ARTICLE AT A GLANCE

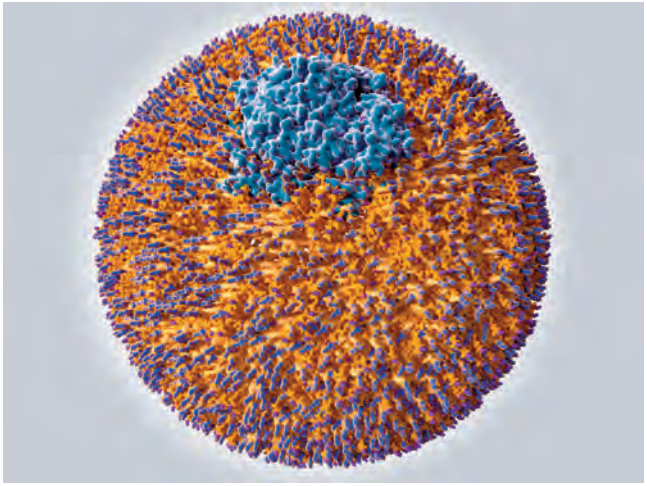
- *Two recent studies have addressed if dietary supplementation with fish oil or DHA-enriched canola oil would modify the plasma levels of PCSK9, a pivotal regulator of LDL levels.*
- *Modest decreases in PCSK9 levels were achieved in both studies, demonstrating that this protein is susceptible to dietary intervention by omega-3 LCPUFA.*
- *New studies may assess if PCSK9 down-regulation by omega-3 LCPUFA supplementation is of benefit to people with elevated LDL levels.*

An elevated plasma low-density lipoprotein (LDL) level is associated with an increased risk of cardiovascular disease. Decades of research and pharmaceutical development have been dedicated to achieving the lowering of the cholesterol content associated with LDL, in the primary and secondary prevention of atherosclerotic cardiovascular disease. Recent efforts to develop LDL-lowering prescription drugs have focused on a new target, PCSK9, or proprotein convertase subtilisin/kexin type 9. PCSK9 is a secreted glycoprotein that regulates the membrane expression of the LDL receptor. This receptor is found within the membranes of cells facing the circulation, and promotes the endocytotic internalization and uptake of LDL lipid cargo into tissues. PCSK9 binds to the LDL receptor and hinders its recycling to the cell surface, decreasing the LDL receptor density available for removal of LDL from the circulation. Therefore, inhibition of PCSK9, or reduction of its levels, leads to a decrease in circulating LDL level by increasing LDL-receptor-mediated LDL clearance. People with loss-of-function mutations in PCSK9 have lower LDL levels and a markedly lower incidence of coronary heart disease. The reverse situation is also recognized: people with gain-of-function PCSK9 mutations have a marked increase in blood cholesterol levels and an increased coronary heart disease incidence.

PCSK9, or proprotein convertase subtilisin/kexin type 9, regulates LDL levels by reducing LDL clearance. Significant interest has developed in recent years to modulate PCSK9 activity and assess its impact, i.e. to lower the risk of developing cardiovascular disease.

LDL levels provide an indication of the excess energy obtained from dietary intake that has been converted to fatty acids and cholesterol by the liver. Cholesterol and fatty acids (as triglycerides) are exported from the liver as VLDL, to distribute triglycerides and cholesterol-fatty acid esters to all peripheral tissues that require these lipids for membrane synthesis, or for storage in the adipose tissue, with the resulting LDL particles being taken up again mainly by the liver. However, in a context of concurrent chronic low-grade vascular inflammation, commonly resulting from a poor diet and a sedentary lifestyle, a sustained oxidative modification of the protein and lipid components that constitute LDL particles can cause the misdirection of LDL lipids into atheromatous sub-endothelial deposits via monocyte/macrophage-mediated retention. Over the course of a decades-long progression of vascular disease, this LDL-associated process is interlinked with pro-inflammatory vascular tissue remodeling and the formation of atherosclerotic lesions.

Although LDL particle levels or LDL-cholesterol estimations in the circulation are increasingly considered a non-causal yet predictive biomarker of cardiovascular disease, interventions that achieve decreases in LDL appear to generally produce a favorable change in the risk of developing atherosclerotic cardiovascular disease. Current recommendations worldwide suggest both lifestyle changes and drug-based intervention to achieve lower LDL levels in people at



risk of atherosclerotic cardiovascular disease. Both pharmacological interventions and lifestyle changes may, however, not achieve the desired targets for LDL reduction, as patient compliance is highly variable and a range of factors determines the individual response to LDL-lowering approaches. Statin therapy achieves reductions in LDL-cholesterol levels through inhibition of the endogenous *de novo* biosynthesis of cholesterol. However, statin therapy also leads to increased PCSK9 levels, which reduce LDL uptake and hepatic clearance - an effect that counteracts its intended pharmacodynamic action. It has been suggested that further LDL lowering beyond that achieved by statins alone may be possible, and that reducing LDL levels below current target levels may offer further cardiovascular disease risk lowering. Newly developed PCSK9 inhibitors have been shown to achieve reduced LDL-cholesterol levels by increasing LDL clearance through removal of the inhibitory activity of PCSK9 on LDL receptor expression. Enhanced LDL clearance by PCSK9 inhibitors is heralded as a breakthrough approach in cardiovascular disease treatment that may eliminate statin resistance, and remove the residual cardiovascular disease risk that cannot be achieved by current LDL-cholesterol-lowering therapies. Modulation of LDL levels remains a major current topic in pharmacology, and understanding the contribution that PCSK9 makes to cardiovascular disease risk is worthy of evaluation. In line with this thinking, serum PCSK9 were recently shown to be associated with the future risk of cardiovascular disease in 60-year old people, independent of typical cardiovascular risk factors such as hypertension, diabetes, smoking, overweight, obesity, and physical inactivity.

Although PCSK9 inhibition has been primarily considered as a promising drug target, its modulation by changes in dietary and lifestyle habits, and the physiological effects of these has been addressed to a very limited extent only. A recent study by Graversen and colleagues at the Department of Cardiology at Aalborg University, Denmark, has carried out a first careful evaluation of the effect of supplemental omega-3 LCPUFA intake on circulating PCSK9 levels in healthy Danish women (age 18 to 70 years). Half of the women were premenopausal (n=46), the other half postmenopausal (n=44). The study is a follow-up analysis of an earlier randomized, controlled, double-blinded study in which the influence of menopause on the distribution of omega-3 LCPUFA into platelets and adipose tissue was addressed. That study had demonstrated that in premenopausal women estrogen levels increased in response to omega-3 LCPUFA intake (2.2 g of omega-3 LCPUFA from fish oil, compared to control oil, daily for 12 weeks). The incorporation of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) into platelet membranes and adipose tissue was nearly identical in premenopausal compared to postmenopausal women supplemented with fish oil. No marked changes in fatty acid levels were found in the control groups.

Two new studies have addressed if omega-3 LCPUFA intake can modulate PCSK9 levels. Dietary approaches to modulate PCSK9 activity have been explored to a limited extent, in contrast to the advanced development of pharmacological approaches that target PCSK9.

Plasma PCSK9 levels were determined by ELISA in pre- and post-menopausal women supplemented with either fish oil or control oil (thistle oil) at baseline and after 12 weeks. Supplementation with fish oil induced a modest decrease in plasma PCSK9 levels that was similar in both pre- and post-menopausal women (a decrease of 29 ng/ml from baseline values of 255 and 286 ng/ml, respectively). In controls, the PCSK9 level did not change over the 12-week intervention period. The authors note that lowering of LDL level was not observed as a result of the interventions. This study shows that in healthy women a decrease in plasma PCSK9 levels can be induced by fish oil intake without any impact on LDL levels. Post-menopausal women did have significantly higher LDL levels at baseline compared to pre-menopausal women (on average 3.03 vs 2.26 mM, respectively). Current target serum levels for treatment of disorders of lipid metabolism are LDL-cholesterol levels <2.6 mM (100 mg/dl), pointing out that the LDL levels in these study participants are in a healthy range and can possibly not be lowered further.

In healthy women, fish oil supplementation for 12 weeks led to a modest reduction in PCSK9 levels. No marked differences were observed between pre- and post-menopausal women.

A recent study by Rodríguez-Pérez and coworkers reported a similar observation from a double-blind randomized controlled trial (COMIT trial) carried out in North-American middle-aged men and women with at least one component of metabolic syndrome (and average baseline LDL level of 3.41 mM). The trial assessed the effect on endothelial function, and plasma fatty acid and sterol profiles, of a 4-week

daily intake of dietary vegetable oil types enriched with omega-3, omega-6 or omega-9 fatty acids. The group that received a DHA-supplemented high-oleic canola oil (DHA as 5% of fatty acids, 36 to 60 g oil per day as a shake-style beverage), had a doubling of plasma DHA level and a reduction in PCSK9 levels of approximately 10%, compared to people receiving canola oil or a high-oleic canola oil. Consumption of the DHA-enriched canola oil also increased HDL cholesterol level and markedly lowered triglycerides (1.30 mM from 1.61 mM). The intervention trial had previously reported an improved blood pressure. Although PCSK9 levels were decreased, no significant enhancement in the lowering of LDL-levels was observed in this study (all tested canola oil types induced a significantly lower LDL level).

In people with some aspect of metabolic syndrome, the dietary intake of a canola oil enriched with DHA led to a modest reduction in PCSK-9 levels over a 4-week intervention period, which could be attributed to DHA.

These new studies show that omega-3 LCPUFA supplementation, as fish oil or DHA, can reduce plasma PCSK9 levels. However, in both studies no differential LDL-cholesterol lowering effect was noted. Future studies will be needed to assess if the modulation of PCSK9 by supplemental omega-3 LCPUFA intake is of relevance to modulating LDL levels in individuals with elevated LDL levels, and in conjunction with other lipid-lowering approaches. PCSK9 not only regulates LDL clearance but is also important for VLDL secretion, a key determinant of triglyceride levels in blood. A recent Mendelian randomization study has provided support for a causal relationship between higher blood concentrations of triglycerides and increased cardiovascular disease risk. Since a well-documented benefit of omega-3 LCPUFA supplementation is the lowering of triglyceride levels, further studies on PCSK9 regulation by increased omega-3 LCPUFA intake will likely provide interesting new insight into the importance of omega-3 LCPUFA status for lipid metabolism and cardiovascular disease risk modification.

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MATERNAL AND INFANT HEALTH

Understanding Defects in Fatty Acid Mobilization to Safeguard Fetal DHA Accretion in Gestational Diabetes

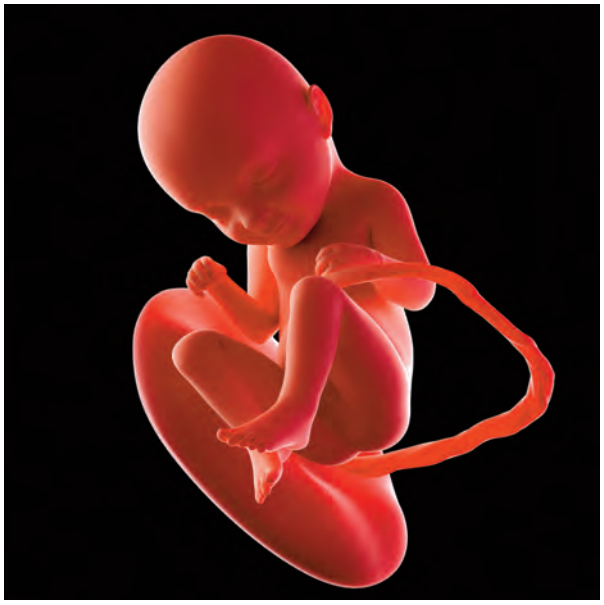
THIS ARTICLE AT A GLANCE

• This study documented the changes in circulating levels of various fatty acids during the third trimester of pregnancy complicated by gestational diabetes.

• In gestational diabetes, defects in the mobilization of several fatty acids were identified, including the DHA necessary for accretion by the fetus.

• In a separate controlled intervention study, fish oil supplementation during the third trimester lowered the incidence of some complications of gestational diabetes in neonates and mothers. Replication in larger studies is necessary to substantiate the findings.

Much of the DHA that a growing fetus needs for its development is derived from the maternal circulation and delivered by maternal-fetal transfer through the placenta. A



notable increase in DHA levels in the maternal circulation occurs during pregnancy, and likely originates from the mobilization of DHA stored in maternal fat deposits and/or from increased DHA biosynthesis, supported by DHA obtained from dietary intake. Fetal DHA accretion during the third trimester increases markedly to support the rapid growth of the central nervous system. Sufficient availability of circulating fatty acids is thus considered to be of vital importance to satisfy the high fetal demands for this polyunsaturated fatty acid (PUFA).

During the third trimester there is an augmented production of placental hormones that play important roles in directing energy reserves towards fetal growth. These increasingly counteract the actions of insulin, in particular placental growth hormone. In pregnant women that have insufficient capacity to produce more insulin, the ensuing insulin resistance is not overcome, leading to an inability to maintain normal blood glucose levels. This temporal condition of glucose intolerance corresponds to a disorder called gestational diabetes. Most frequently, gestational diabetes is transient and disappears after birth. Gestational diabetes is also considered a pre-diabetic condition, and mothers who displayed diabetes during pregnancy are routinely checked for diabetes some six weeks after giving birth.

The prevalence of gestational diabetes varies considerably depending on the criteria used for screening and diagnosis, but all rely on diagnostic glucose thresholds. Diagnostic glucose thresholds are glucose limits that are surpassed in the diabetic condition after testing glucose levels upon taking an oral bolus intake of glucose. Current diagnostic criteria used by different national and international organizations are not harmonized. Using thresholds proposed by the International Association of Diabetes in Pregnancy Study Groups (IADPSG), some 18 percent of pregnant women in different centers globally are diagnosed with gestational diabetes. The prevalence of gestational diabetes also varies strongly among countries and populations (ranges from ~8% to 26% gestational diabetic pregnancies are described). Diabetes can have its onset during pregnancy, but may also be already present before pregnancy (pre-gestational diabetes). Along with the growing prevalence of type 2 diabetes and obesity, a significant increase in both pre-pregnancy overt diabetes as well as gestational diabetes prevalence has been noted in recent years.

Gestational diabetes develops in an important percentage of pregnancies, and has potentially severe consequences for both the developing fetus and the mother.

Gestational diabetes can have significant effects on maternal and fetal health. A fetus in a diabetic mother will be exposed to higher concentrations of glucose, since glucose is transferred across the placenta by facilitated diffusion, and such fetuses are often born with excessive adiposity and birthweight (macrosomia, "large for gestational age"). Hyperglycemia in the fetus can also lead to polyuria leading to polyhydramnios (excess amniotic fluid), as well as cardiac and other malformations. A high birthweight during birth is likely to cause physical injuries to both mother and baby, resulting in an increased need for Cesarean section. After birth, there is a risk of hypoglycemia in newborns. Improved management of gestational diabetes is therefore of significant value to better infant and maternal healthcare.

Recent studies have indicated that in addition to alterations in metabolic regulation, the materno-fetal transport of DHA is disturbed in women with gestational diabetes, even if treated with insulin. The relative abundance of DHA within lipids in placenta and cord blood is inversely related to fetal adiposity. Given that macrosomia may even occur in pregnancies with well-controlled gestational diabetes, guaranteeing sufficient fetal accretion of DHA may be very important for proper fetal development and neonatal outcome in all cases of gestational diabetes. Although reductions in the placental transport of DHA have been observed in gestational diabetes, it is not known whether the increased provision of DHA available for maternal-fetal transfer across the placenta may already be altered in gestational diabetes. In order to better understand how mobilization of maternal long-chain PUFA is regulated during the third trimester, Zhao and colleagues measured the changes in the circulating levels of DHA and a range of other fatty acids in healthy pregnant women and compared these with women with gestational diabetes. The research was carried out by researchers at the Department of Obstetrics and Gynecology, Sainte Justine Hospital Research Centre, University of Montreal, and colleagues at several other departments at University of Montreal, the University of Sherbrooke, Laval University, Canada, and the Shanghai Jiao-Tong University School of Medicine, China.

For this study, 140 women with singleton pregnancies were selected at 24 to 28 weeks of gestation. Women with pre-gestational diabetes were excluded. Of the selected women, 24 developed gestational diabetes (diagnosed according to the criteria of the American Diabetes Association using the 75-g 2-hour oral glucose tolerance test, with two of three glucose values exceeding the following cut-off – fasting: 5.3 mM, 1 h: 10 mM, and 2 h 8.6 mM). There were no significant differences between the two study groups with respect to race, age (average of 32 years old), pre-pregnancy BMI, and smoking and alcohol use characteristics. The newborns born in the two groups of women displayed no differences in average weight, but the average length of gestation was somewhat shorter (less than 1 week) and the birthweight *z* score was higher for babies born to women with gestational diabetes. Dietary intake was determined using a food-frequency questionnaire during weeks 24-28. No significant differences in macronutrient or fatty acid intake were noted between the two study groups.

Deficiencies in the fetal accretion of DHA may occur in gestational diabetes. This study assessed if defects in the mobilization of maternal fatty acids that are needed for proper fetal development occur during gestational diabetes.

Fatty acid concentrations in the maternal circulation were measured in plasma total lipids by direct acid-catalyzed transmethylation and gas chromatography (notably, a widely-employed derivatization method was used that had been developed 30 years earlier at this same institution). In women with an uncomplicated pregnancy, a range of fatty acids increased in concentration from week 24-28 to 32-35. These included several saturated and monounsaturated fatty acids, and among the PUFA, linoleic acid (LA), arachidonic acid (AA), alpha-linolenic acid (ALA), and DHA levels were increased (**Table 1**). No changes were observed in the levels of ɣ-linolenic acid, docosapentaenoic acid n-3 (DPA n-3), and eicosapentaenoic acid (EPA). Interestingly, plasma levels of several of the fatty acids that had increased during the third trimester in healthy pregnancies did not increase, or significantly less so, in the diabetic women. These were ALA, DHA, dihomo-ɣ-linolenic acid (20:3 n-6), docosatetraenoic acid (omega-6) and palmitoleic acid (see **Table 1**). Although not all observed increases were of a large magnitude, clear differences in fatty acid mobilization between women with a healthy pregnancy and those with gestational diabetes were observed.

At study baseline (week 24-28), the average concentrations of all measured fatty acids were the same in both study groups, indicating that differences in measurable fatty acid mobilization occurred after week 24-28 of gestation. Only one exception was identified: the baseline concentration of the saturated fatty acid eicosanoic acid (20:0) was approximately 9% lower in diabetic women. Although similar increases in the plasma level of this fatty acid were observed in weeks 32-35 in both normal and diabetic pregnancies, its level remained -10% lower in diabetic women throughout the third trimester. During the third trimester the indexes of the Δ6-desaturase and

Table 1. Change in fatty acid concentration (micromolar) in maternal plasma from weeks 24-28 to 32-35

Fatty acid	Uncomplicated pregnancies	Gestational diabetes
Linoleic acid	862 ± 96 *	937 ± 222 *
Dihomo-gamma-linolenic acid	17 ± 5 *	-12 ± 14
Arachidonic acid	66 ± 13 *	123 ± 33 *
Docosatetraenoic acid	3.4 ± 1.0 *	-2.3 ± 1.9
Total omega-6 PUFA	948 ± 105 *	1041 ± 256 *
Alpha-linolenic acid	42 ± 6 *	28 ± 16
Docosahexaenoic acid	33 ± 7 *	14 ± 13
Total omega-3 PUFA	75 ± 13 *	13 ± 16 *
Palmitoleic acid	125 ± 26 *	14 ± 31

*Values indicated are means ± S.D. *Significant increase; P<0.01*

stearoyl-CoA-desaturase enzyme activities (estimated from the ratios of fatty acid product to substrate abundance in plasma lipids) significantly decreased in gestational diabetes.

The results of this study indicate that in women with gestational diabetes, a physiological increase in blood levels of several PUFA, notably ALA and DHA, during the third trimester of pregnancy, is disturbed. Although statistically significant, the relative and absolute changes are not very marked: the increases in ALA and DHA concentrations were 42 and 33 µM (respectively), whereas they were 28 and 14 µM (respectively) in women with gestational diabetes, on a background of baseline levels of ~160 and 390 µM, respectively. The results point to the possibility that lower amounts of specific fatty acids may be available for maternal-fetal transfer across the placenta in gestational diabetes. It remains to be demonstrated if the uncovered defect under conditions of diabetes is due to a reduction in the biosynthesis of specific lipids, insufficient mobilization from storage lipids, or enhanced metabolic degradation of these fatty acids. It remains to be addressed by means of a targeted analysis of lipid species whether the observed changes in these circulating fatty acids may correspond to a specific lipid type involved in maternal-fetal transport of LNA and DHA, *e.g.* esterified within lysophospholipids, which are a substrate for active transport by Mfsd2a, or as free fatty acids, by facilitated diffusion. The researchers point out that a controlled clinical study is needed to show whether pregnant women with gestational diabetes may potentially receive benefit from supplemental intake of essential fatty acids, in particular DHA.

A small double-blind randomized placebo-controlled trial in pregnant women with gestational diabetes assessed if supplementation with fish oil during the third trimester might be of benefit to neonatal and maternal outcome.

A recent intervention trial had independently completed an assessment of the potential beneficial effects of supplemental omega-3 LCPUFA during the third trimester on maternal and neonatal health in gestational diabetes. In a double-blind randomized placebo-controlled clinical trial, Jamilian and colleagues addressed the effect of omega-3 supplementation on gestational diabetes outcome in a group of pregnant Iranian women. The study was carried out at the Department of Gynecology and Obstetrics at Arak University of Medical Sciences, Iran, together with colleagues from Kashan University of Medical Sciences, Iran, and the Department of Medicine at the University of Alberta, Edmonton, Canada. Fifty-six women diagnosed with gestational diabetes at week 24-28 of gestation were randomly assigned to one of two dietary supplements: a treatment group (n=27) that took daily 1 gram encapsulated fish oil containing 180 mg EPA/120 mg DHA, and a control group (n=27) that received an identically-appearing placebo (liquid paraffin). The intervention period was six weeks. Stratification based on BMI and weeks of gestation (less or more than 26 weeks) was used for randomization allocation.

The women (18-40 years) all had a singleton pregnancy, did not take oral hypoglycaemic agents, and were diagnosed with gestational diabetes using the 2-h 75 g oral glucose tolerance test. Women with pre-gestational diabetes, chronic medical conditions, special dietary needs, or who required insulin therapy during intervention were excluded from this study. All women received folic acid and iron supplementa-

tion, received education on healthy eating habits, and were advised to maintain their normal routines. Compliance was promoted by cell phone text reminders, and was assessed by capsule counts. Dietary intakes and physical activity were monitored throughout the study. Daily nutrient intakes were calculated from three-day food diaries and calculated with dedicated software adapted for Iranian foods.

The primary outcomes of the study were several indicators of maternal and infant health: polyhydramnios, pre-eclampsia, gestational age, Cesarean section, and newborn's size, Apgar score, and hyperbilirubinemia. Secondary outcomes were levels of circulating markers of inflammation and oxidative status. At the beginning and end of the study, plasma and serum samples were collected, and body weight and height were determined from which BMI was calculated. Compliance was reported to be very high.

No significant differences in a number of general characteristics (age, height, weight, change in weight, BMI and gestational age of the women) between the two groups were identified at baseline or at the end of the study period. No differences in mean dietary intake throughout the study of macronutrients, PUFA, and several micronutrients were identified. Women that had received supplementation with omega-3s did not develop polyhydramnios or need hospitalization, compared to 11% of the control group. In newborns from women who received the supplemental fish oil for six weeks, hyperbilirubinemia incidence and hospitalization rate was lower with 7.7%, compared to 33% of neonates for both outcomes in the control group. No differences were found for gestational age, newborn weight or ponderal index, and Apgar scores. Reductions in the need for Cesarean section, need for insulin-therapy, macrosomia, weight and length were not statistically significant. Analysis of plasma and serum markers of inflammation revealed that fish oil supplementation abrogated an increase in C-reactive protein that occurred during gestational diabetes. Omega-3 supplementation was also reported to reduce the levels of malondialdehyde in plasma, an indicator of thromboxane synthase activity as well as non-enzymatic lipid peroxidation.

The studies indicate that alterations in circulating levels in several fatty acids developing during the third trimester in women with gestational diabetes may compromise fetal development. Supplementation with fish oil may counter some of the negative consequences of gestational diabetes.

The results of this controlled intervention study suggest that daily supplementation of pregnant women with gestational diabetes in the third trimester with a relatively small daily dose of EPA/DHA from fish oil may bring about improvements in several aspects of infant and maternal health. In this relatively small study this was mainly shown as lower needs for hospitalization of both newborns and mothers, as well as absence of polyhydramnios and lower incidence of neonatal hyperbilirubinemia. Total incidence numbers were low for a number of outcomes and future larger studies are needed to confirm the findings, and provide support for some of the reductions in complications that were not conclusively identified in this study. A recent meta-analysis did reach a similar conclusion that omega-3 supplementation during the third trimester can reduce hyperbilirubinemia and hospitalization rate in newborns, and reduced CRP levels in women with gestational diabetes.

There is now supportive evidence of defects in DHA provision to the fetus in women with gestational diabetes. It is not known if gestational diabetes interferes with the provision of other fatty acids that are needed by the fetus, but additional fatty acids are mobilized. Omega-3 LCPUFA supplementation during the third trimester may improve neonatal liver function, has an anti-inflammatory effect in women, and may support a generally improved clinical outcome in both infants and mothers, given reduced hospitalization rates. Larger studies are needed to confirm these findings, and determine if all of the essential fatty acids that are mobilized during the third trimester are important for optimal fetal development. Intervention studies will then be able to inform us if defects in fetal fatty acid accretion that may develop during gestational diabetes can be compensated by supplementation of the mother. A previous study that had studied the effect of a DHA-enriched omega-3 supplement intake from 21 weeks onwards did not find a preventive effect on the incidence of gestational diabetes. This suggests that supplementation with omega-3 LCPUFA may address some of the effects of gestational diabetes, but not the underlying disorder itself.

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IMMUNE FUNCTION

Regulation of B-cell IgE Production by a DHA-Derived Lipid Mediator

THIS ARTICLE AT A GLANCE

• This study assessed the effect of DHA-derived lipid mediators on IgE formation by B-lymphocytes, an important component of the adaptive immune system that is activated in allergies.

• The study reports that resolvin D1 inhibits the differentiation of human B-cells to an IgE-producing type by inhibiting B-cell isotype class-switching.

• The results of this study point to the activation of a new mechanism of anti-inflammatory action of omega-3 LCPUFA, and opens up research possibilities into modulating IgE-mediated allergic reactions in a novel way.

Immunoglobulin ε (IgE) is the central antibody mediator of the allergic response. Allergic responses are a type of localized inflammatory response (also called a localized anaphylactic or immediate hypersensitivity reaction) that are operative in tissues directly exposed to the external environment, such as the skin, nose, eyes, airways, throat and intestinal tract. Allergic responses occur upon exposure to allergens, which are usually proteins from non-mammalian multicellular organisms such as insects, arachnids (mites and spiders) and plants (pollen). The well-known symptoms of allergy, itch and irritation, mucous secretion, changes in respiratory pattern, and enhanced blood flow, contribute to the physical and chemical removal of the offending agent (through scratching, removal by mucous transport and tear formation, coughing, swallowing, and proteolytic degradation). Allergies can also develop towards a diverse array of man-made organic and inorganic substances, components of food, and to physical stimuli such as elevated or low temperatures, light, and dry air, each of which may involve IgE to a variable extent. In chronic and exacerbated forms of allergy, such as eczema, rhinitis, and allergic asthma, reactions are considered pathological and have a significant disabling effect on life quality. Severe acute allergic responses that involve a generalized involvement of several organs are called systemic anaphylactic reactions, and can be life-threatening. Allergic reactions and diseases are very common, affecting at least 1 in 10 people.

Why allergies develop in the first place is often unclear, but the extent of an allergic response to any specific allergen appears to be determined by the level of desensitization that may have occurred upon prior contact to low levels of allergenic exposure. Early life exposure (*in utero* or during childhood) informs the body about the presence of allergens as proxies for specific other organisms that are present in the natural habitat and food of the exposed person. Such early exposures are believed to trigger tolerance, *i.e.* a much reduced or absent immune response towards those allergens that an individual is and will normally be exposed to. Exposure later in life to an allergen that has never been previously encountered can rapidly lead to sensitization. Sensitization involves the development of B-lymphocytes producing IgE antibodies that can selectively bind to the specific allergen. IgE dimers stably bind to high-affinity IgE receptors on mast cells (present in all superficial tissues) and are activated upon interaction with allergen, triggering mast cell degranulation and the release of vasoactive and neuroactive mediators that bring about the symptoms of allergy.

People with allergies are characterized by increased levels of IgE in the circulation, pointing at a generalized increased IgE production. People with a genetic predisposition to produce IgE towards allergens, *i.e.* having atopy, will have a higher risk of developing allergic disorders, and can be affected already at early stages in infancy by atopic dermatitis, progressing to allergic rhinoconjunctivitis and asthma later in childhood. Reducing IgE levels in blood and tissues with monoclonal antibodies is an effective approach to reducing some forms of allergy. Widely used allergy medications attempt to make mast cells less sensitive to activation by allergen-IgE complexes, or interfere with the activity of the allergic mediators produced by mast cells (such as histamine or cysteinyl-leukotrienes).

Omega-3 LCPUFA play important roles in regulating immune responses. The formation and action of specific biologically active oxygenated derivatives of EPA and DHA

Allergies occur in more than 1 in 10 people. The management of allergies requires several approaches to keep symptoms under control. How PUFA may help to prevent or treat allergies is still being investigated.

contribute to the regulation of different aspects of the anti-inflammatory and inflammation resolution-promoting activity of EPA and DHA. Many aspects of the innate arm of the immune system have been identified to be modulated by EPA- and DHA-derived lipid mediators. More recently, specific elements of the adaptive immune system have also been documented to be under the influence of omega-3 LCPUFA-derived mediators, which include the regulation of dendritic cells, and the promotion of B-cell IgG and IgM formation. An EPA-derived lipid mediator, 17,18-epoxy-tetraenoic acid (17,18-EpETE), was identified recently and found to exert anti-allergic activity in a murine model of food allergy, acting at least in part through reducing mast cell activation. It is still unknown if IgE formation, as an important component of the adaptive immune system, may be regulated by omega-3 LCPUFA or by any of the bioactive omega-3 LCPUFA-derived lipid mediators.

In order to evaluate if IgE-mediated responses may be modulated by omega-3 LCPUFA, a recent investigation has screened several DHA-derived resolvins for their activity on IgE formation by human IgE-producing B-lymphocytes. The study was performed by Kim and colleagues from the Department of Microbiology and Immunology, at the University of Rochester, NY, USA, in collaboration with colleagues in the departments of Pulmonary and Critical Care, and Environmental Medicine, at the same university. First, B-cells were isolated from human blood, and experimental conditions were optimized to identify the conditions under which these cells switched to primarily produce IgE. B-cells can differentiate into antibody-secreting cells when stimulated by the CD40 ligand (a protein secreted by platelets when activated) and activation of a pattern recognition receptor (such as the toll-like receptors (TLRs), which are receptors that recognize conserved molecular domains of microbes and parasites). High and predominant IgE-secretion was achieved by exposing the cells to a mixture of a TLR-9 agonist, interleukin-4 and CD40 ligand.

This study identifies a DHA-derived endogenously formed lipid mediator that can regulate IgE formation, and elucidates a mechanism of action. This is an important observation since it provides insight into the potential anti-allergic activity of omega-3 LCPUFA.

17-Hydroxy-docosahexaenoic acid (17-HDHA) and resolvin D1 reduced IgE release in a concentration-dependent manner (reductions were observed at 10 and 100 nM, with test substances added to the cells every day for a period of six days). Under the experimental conditions tested, the inhibitory effect was specific for IgE, and no effect on IgM or IgG production was found. RvD2 and the RvD1 epimer AT-RvD1 did not reduce IgE production. The reduction in IgE production was related to a decrease in the number of IgE-producing B lymphocytes. This observation suggested that the inhibitory effect was not on actual IgE formation or secretion but at the level of differentiation of B-lymphocytes into cells that were able to produce IgE, *i.e.* a change in the immunoglobulin class-switching of precursor B-cells to IgE-producing B-cells.

For class-switching to occur to the IgE antibody isotype, the upregulation of a specific epsilon germline transcript (εGLT) is necessary, which is promoted by the T-helper cytokines IL-4 and IL-13, and the CD40 ligand. The researchers found that εGLT transcription was induced very rapidly after exposing the B-cells to the inducing conditions. RvD1 markedly reduced this transcriptional upregulation of εGLT, while not affecting the transcription of other factors that might be involved in the isotype switching process (activation-induced cytidine deaminase, CD23, and the receptor for IL-4).

Since εGLT is under the control of the transcription factor STAT6, the researchers next assessed whether RvD1 interfered with activation of STAT6 by assessing its phosphorylation and intracellular translocation into the nucleus (where it acts to regulate DNA transcription). B-cell stimulation induced a rapid phosphorylation of STAT6 and relocation to the nucleus, but both processes were not affected by RvD1. However, RvD1 pretreatment markedly reduced the binding of STAT6 to the promotor region of εGLT. The researchers evaluated the possibility that the transcriptional repressor Bcl-6 (B-Cell Lymphoma 6), known to counter-regulate STAT6 transcriptional activity, could be the point of regulation by RvD1. Although B-cell stimulation led to a small increase in Bcl-6 expression, this was not affected by RvD1. RvD1 did stimulate a marked increase in Bcl-6 protein levels, indicating that RvD1 activated translation of Bcl-6 messenger RNA to protein, or stabilized the transcribed Bcl-6 protein. In summary, the

DHA-derived lipid mediator RvD1 reduces B-cell differentiation into IgE-producing cells by increasing the levels of the transcriptional repressor Bcl-6 that blocks STAT6-activated transcription of the εGLT transcript, necessary for immunoglobulin isotype switching.

Several observational studies have suggested that omega-3 LCPUFA intake and supplementation can lower the symptoms of allergic and asthmatic disease, but carefully controlled intervention studies are lacking and a mechanism of action had been unclear. In a recent double-blind crossover study in mild-moderate asthmatics, an increased dietary intake of EPA/DHA did not provide a beneficial effect on asthmatic symptoms towards a mannitol challenge (a direct activator of mast cells), even though several EPA- and DHA-derived lipid mediators were confirmed to be elevated in serum compared to control. The formation of DHA-derived (D-series) resolvins was not determined in the study, but observing no effect of increased omega-3 LCPUFA intake on the airway constriction triggered by direct mast cell activation and bypassing an IgE-mediated component, may suggest that omega-3 LCPUFA could exert a more pronounced modulatory action upstream of the mast cell response. Another study that has assessed the effect of omega-3 LCPUFA supplementation (900 mg daily from fish oil) during pregnancy on allergy incidence in infants found that eczema and sensitization to egg were reduced during the first year of life.

The study indicates that the DHA-derived lipid mediator resolvin D1 can reduce IgE formation in human B-lymphocytes through a unique inhibitory action on the class-switching of B-cells towards IgE-producing B-cells, effectively reducing the number of B-cells that can produce IgE.

New studies aimed at addressing the beneficial role of essential fatty acids in allergic disorders now have a strong indication to focus on the role of RvD1 (as well as the pathway intermediate 17-HDHA) as a selective, or perhaps specific, endogenous DHA-derived lipid mediator that can influence IgE formation through inhibiting B-cell isotype switching. A novel approach to reduce IgE formation through the action of specific DHA-derivatives is of significant interest to better understand the role of DHA intake in the modulation and prevention of allergic disease, as well as the development of new anti-allergic compounds based on the RvD1 structure.

The results of this study provide insight into a mechanism whereby omega-3 LCPUFA may regulate inflammation, namely by activating the transcriptional repressor Bcl-6. Bcl-6 is known to counter-regulate and inhibit transcriptional programs activated by NF-*k*B, one of the central regulating systems involved in activating innate immune responses. Evaluation of the scope of the involvement of Bcl-6 in different immune cell types will likely provide a more profound understanding of the influence of omega-3 LCPUFA on various aspects of immune regulation and inflammation control.

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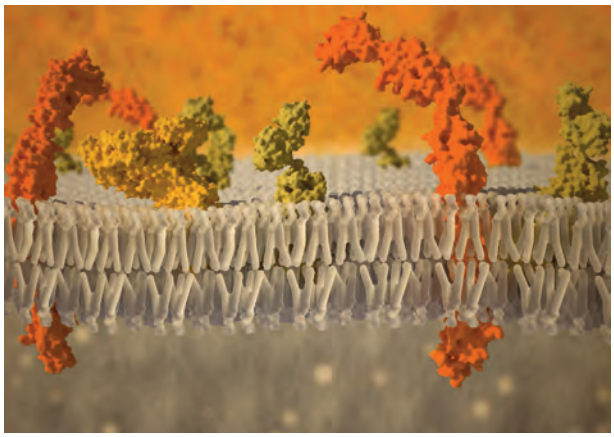
Making PUFAs Available for Tissue Protection – Selective Phospholipase-Mediated Membrane Release of Omega-3 LCPUFA

THIS ARTICLE AT A GLANCE

- *A recent study in mice reports that human and mouse secretory group X phospholipase A_2 preferentially releases omega-3 long-chain polyunsaturated fatty acids to serve anti-inflammatory and tissue-protective activities in the colon.*
- *Selective release of arachidonic acid by a different phospholipase, coupled to the biosynthesis of prostaglandin E_2, occurs in parallel, and also contributes to the tissue-protective activity.*
- *Growing recognition of the involvement of distinct phospholipases in specific immunological events may one day allow us to better support their activities to drive beneficial outcomes.*

In general terms, polyunsaturated fatty acids (PUFA) serve two purposes in animal physiology: they have structural roles as part of lipid membranes thereby influencing membrane biophysical properties (by modulating its fluidity, membrane domain formation, and interactions with proteins that are



embedded within or interact with membranes), and as substrates for the formation of an array of signalling molecules (lipid mediators) that regulate the activity of virtually all cell types in the body. For this second function, PUFA need to be first released from the membranes. The release of PUFA is initiated upon a cell receiving a stimulus, and is driven by specialized enzymes called phospholipases that have the ability to interact with membranes and catalyze the hydrolysis of fatty acids from membrane phospholipids. Many phospholipase isoenzymes are known, each with its own specific cellular and subcellular expression profile, substrate preference for the lipids they recognize and the fatty acids they release from these, and sensitivity to a variety of factors that regulate their enzymatic activity (such as pH, calcium level, phosphorylation state, and the properties of the membranes on which they act).

One phospholipase enzyme, the cytosolic phospholipase A_2 ($cPLA_2$ or group IVA PLA_2), has long been regarded as an important phospholipase that releases arachidonic acid (AA) from membranes. This occurs after a cell is stimulated (*e.g.* by exposure to a specific hormone, or upon recognition of a microbe in the case of an immune cell). After $cPLA_2$ activation, the free fatty acids formed are further metabolized by different oxygenases that "couple" with phospholipase-mediated release to form fatty acid metabolites, also called lipid mediators, each with specific biological activities. The precise sum of lipid mediators that is formed by this combination of phospholipase and biosynthetic enzymes depends on which enzymes are expressed in the vicinity of the site of PUFA release. Stimulus-coupled AA-release for eicosanoid (AA-derived lipid mediators) generation generally refers to an intracellular event. The lipid mediators that are produced are frequently exported/secreted and exert potent extracellular modulatory and signalling functions in the tissue where they are produced. They act as locally acting hormones (autacoids) with very short half-lives that modulate the function of cells within the organ where they are formed, carrying information on cellular status. For example, an immune cell may sense an infectious bacterium, triggering phospholipase-mediated release of AA, which is transformed into an eicosanoid that activates another cell to act against the bacterium by secreting an anti-microbial peptide. In addition to intracellular phospholipases, several secretory phospholipases secreted from cells have extracellular actions. One of these is group X sPLA2 (sPLA2-X/sPLA2G10). sPLA2-X hydrolyzes AA from zwitterionic membrane phospholipids. Studies have revealed that sPLA2-X has anti-viral and anti-bacterial activity, can hydrolyze platelet-activating factor (an im-

For PUFA such as EPA, DHA and arachidonic acid to act as substrates for the formation of signalling substances, they first need to be released from phospholipid stores within cell membranes. This reaction is catalyzed by phospholipases, of which many types are known.

portant pro-inflammatory mediator), and also releases AA from intracellular and extracellular membrane sites for the formation of eicosanoids. Several studies have implicated a role for sPLA2-X in different inflammatory settings.

It is recognized that selectivity in the release of different PUFA types by specific phospholipases functions in combination with stimulus-dependent coupling of such release with particular lipid mediator biosynthetic enzymes. Additionally, some phospholipases have in recent years been recognized to selectively release omega-3 long-chain polyunsaturated fatty acids (LCPUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to enable their transformation into specific bioactive lipid mediators. The group IID secretory PLA_2 (sPLA2G2D) was documented not long ago to selectively release AA for the formation of a specific prostaglandin D_2-derived anti-inflammatory metabolite (15-deoxy-Δ12,14-prostaglandin J_2), alongside the release of DHA to favor the formation of the inflammation-resolving autacoid resolvin D1. These lipid mediators were found to contribute to protection against experimental contact dermatitis and the control of dendritic cell activity. In a similar fashion in murine skin, group IIF PLA_2 (PLA2G2F) preferentially releases DHA to drive the formation of protectin D1, another DHA-derived pro-resolving lipid mediator.

This study is the first to show in vivo that the secretory group X phospholipase A2 is activated in inflammatory conditions to selectively release EPA/DHA to mediate protective effects in the colon. Both the human and mouse enzyme appear to share this ability.

A recent study has now uncovered a new role for the group X $sPLA_2$ in the release of omega-3 LCPUFA coupled to the formation of lipid mediators that serve an anti-inflammatory role in the colon of mice. The study by Murase and colleagues was carried out at the Tokyo Metropolitan Institute of Medical Science, with colleagues at the Department of Pharmacy at Showa University, and various other institutions in Tokyo, Japan. The implications of this recognition are likely to be profound since the results suggest that membrane pools of specific PUFA are accessible selectively to specific phospholipase enzymes as important points of control towards regulating specific cellular functions.

First, the researchers carried out a detailed characterization of the immune function and anatomy of a mouse that carried a transgenic construct of the human group X $sPLA_2$. These mice overexpressing the enzyme had a smaller heart, and a smaller spleen with reduced white pulp areas (sites of lymphocyte development), containing fewer B-cells with a different stage of differentiation than splenic B-cells in normal wild type mice. Serum levels of the IgG1 and IgE antibody types were increased. T-lymphocytes were less mature than in wild type mice, and the levels of T-lymphocytes in the circulation were markedly reduced. In the thymus, several genes that are involved in lymphocyte maturation, proliferation and migration were expressed at lower levels. Generalized overexpression of transgenically-expressed human group X $sPLA_2$ in mice thus led to an altered immune phenotype.

Interestingly, the immune status of resident macrophages in the abdominal cavity was found to be highly polarized to an anti-inflammatory phenotype. When the mice were stimulated to mount an acute inflammatory response, a marked inhibition of inflammation was observed (approximately 75% reduction in macrophage infiltration). Furthermore, macrophages isolated from the bone-marrow displayed an anti-inflammatory phenotype upon stimulation. In addition to the lymphocyte immunosuppression and the anti-inflammatory polarization of macrophages, the mice were found to be leaner than wild type mice, and had reduced visceral and subcutaneous adipose tissue.

In order to understand how overexpression of human group X $sPLA_2$ could confer such an anti-inflammatory phenotype to mice, the investigators measured a range of lipid mediators derived from the PUFA that $sPLA_2$ might be releasing from cellular membranes. In the spleen, the enhanced release of AA, EPA and DHA was confirmed. Only small non-significant increases in AA-derived eicosanoids were found, but the formation of several EPA- and DHA-derived mediators was much greater than in control mice. In the skin, there was increased release of DHA and formation of DHA-derived protectin D1. And in colonic tissue, there was no change in the formation of AA-derived lipid mediators, but a significant increase in EPA-derived 12-HEPE. Taken together, the results in this mouse model suggested that human group X $sPLA_2$ is able to mobilize omega-3 LCPUFA, in addition to and possibly more efficiently than AA, and appeared to direct these PUFA towards the formation of various anti-inflammatory autacoids.

The researchers next turned to studying the role of the endogenous murine group X phospholipase A_2 homologous enzyme, sPLA2-X, in wild type mice and in mice lacking this enzyme. Several types of phospholipase A_2s were found to be naturally expressed in mouse colon, among which sPLA2-X. After inducing experimental colitis (by introduction of a strong irritant

in the drinking water), a more marked reduction in body weight over a 1-week period was found in mice that lacked sPLA2-X compared to wild type mice. Diarrhea and blood present in feces was significantly worse in mice with colitis when the enzyme was absent. The protective effect of sPLA2-X was also seen upon histological examination with reduced epithelial damage, ulceration and submucosal inflammation in mice that had normal sPLA2-X expression compared to the knock-out mice. Deficiencies in any of the other phospholipase enzymes normally found in the colon did not confer this susceptible phenotype in the colitis model. Mice that lacked sPLA2-X and were challenged by experimental colitis displayed a marked upregulation of several cytokines, chemokines and macrophage markers, all associated with a marked pro-inflammatory reaction. Protection from colitis was subsequently shown to be due to contributions from sPLA2-X expressed in colonic epithelium. An additional contribution from sPLA2-X present in hematopoietic cells furthermore appeared to contribute to tissue protection, particularly for other effects such as a splenic response to injury and the maintenance of hematocrit.

Analysis of endogenously produced lipid mediators during colitis showed that mice with normal sPLA2-X expression displayed production of higher levels of several EPA- and DHA-derived lipid mediators with pro-resolving activity (18-HEPE, resolvin D2, 4-HDHA and 7-HDHA) than knock-out mice. The formation of several prostaglandins, thromboxane and 12-HHT, all AA-derived lipid mediators, was however unaltered in mice lacking sPLA2-X. The release of EPA, docosapentaenoic acid (DPA) omega-3 and DHA was significantly higher in wild type mice with colitis than in the sPLA2-X knock-out mice, but the release of AA was increased to a much smaller extent.

Next, the authors tested if the administration of various PUFA and several select lipid mediators could modify the inflammatory responsiveness of lymphocytes isolated from colonic tissue from wild type mice with colitis. All tested PUFA (AA, EPA, DPA and DHA) reduced the secretion of pro-inflammatory cytokines. The anti-inflammatory effect on lymphocytes was stimulated by activation of the GPR120 receptor, but was not activated by some of the downstream lipid mediators generated from EPA or DHA (at least not by the resolvins that were tested). Daily rectal installation of EPA and DHA reduced inflammation and restored body weight loss in mice lacking sPLA2-X with colitis. Taken together, the results suggest that sPLA2-X may play an important role in mediating the release of omega-3 LCPUFA to mediate anti-inflammatory and tissue protective actions. On lymphocytes these anti-inflammatory actions may be mediated directly by the free fatty acids. The authors did consider that some of the detected omega-3 LCPUFA-derived lipid mediators may be exerting tissue-protective and anti-inflammatory actions on immune cell types other than lamina propria lymphocytes, but this was not tested.

The study addresses some additional points, including a demonstration that selective release of AA by cPLA$_2$ and subsequent formation of prostaglandin E$_2$ (PGE$_2$) occurs in parallel to the uncovered action of sPLA2-X, and contributes to the tissue protective response towards colitis. The overall conclusion of the study is that cPLA$_2$ and sPLA2-X exert a protective effect against colitis by facilitating the formation of distinct lipid metabolites derived from AA and from omega-3 LCPUFA (EPA, DPA and DHA), respectively. In addition to the colon, sPLA2-X was found to be expressed in the sperm cell acrosome, where the enzyme selectively liberated DPA omega-3 and DHA from the sperm cell membrane after capacitation (activation of sperm cells permitting ovum fertilization).

The study also reveals a potentially critical role of sPLA2-X in sperm cell fertility, as it mediates the selective release of DHA and DPA during the acrosomal reaction, and DPA can restore the loss of fertility in sperm cells that lack this enzyme.

This study offers a fundamental insight into the role of specific phospholipases in being able to selectively release specific PUFA species and couple this release to important downstream functions, such as shown here in anti-inflammation, tissue protection, and sperm cell fertility. This study supports the notion that substrate specificity (fatty acid selectivity) of a phospholipase is possibly even more important than the absolute and relative content of EPA/DHA versus AA bound within membrane phospholipid pools, on the premise that sufficient PUFA substrate is available, to appropriately couple the release to cell-type specific downstream lipid mediator formation and action. In previous studies sPLA2-X was shown to play an aggravating role and also an inhibitory role in the development of atherosclerosis. It now remains to be determined if such divergent outcomes depend on the relative availability of EPA/DHA versus AA in the membranes on which sPLA$_2$-X acts. An anti-inflammatory role for the human sPLA$_2$-X in macrophages had been previously reported, and although release of omega-3 LCPUFA-derived lipid mediators was not determined in that study, the enzyme was able to couple AA release to the formation of AA-derived lipid mediators with anti-inflammatory activity. Although further research is needed to demonstrate the importance of the relative avail-

ability of EPA/DHA versus AA availability, this observation may mean that the same enzyme is able to also use AA and couple this release to specific AA-derived lipid mediators that also mediate an anti-inflammatory activity.

This emerging appreciation of selectivity in the release of PUFA by distinct phospholipases in combination with downstream stimulus-dependent coupling towards specific lipid mediator profiles provides us with a reminder that PUFA biology is highly regulated and complex. Making careful assessments of the influence of both omega-3 and omega-6 status of a cell or tissue, the involvement of distinct phospholipases, and the panel of mediators formed and that activate specific cellular reactions, will over time provide a better understanding of the programs that our biological system employs to regulate defined processes in physiology.

Several examples now exist to support the idea that specific phospholipases appear to be capable of selectively releasing omega-3 LCPUFA and coupling this release to the formation of tissue-protective anti-inflammatory lipid mediators.

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Indications of an Association between Higher PUFA Intake with Improved Lean Mass and Reduced Adiposity in Children

THIS ARTICLE AT A GLANCE

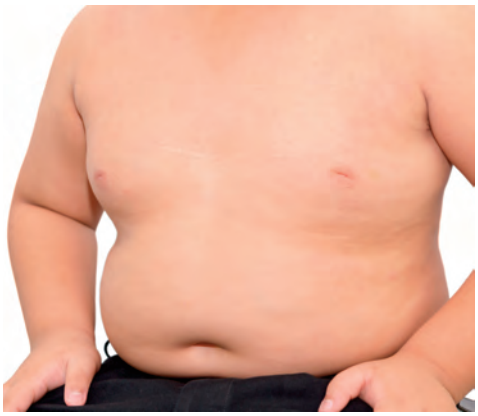
- *This study addressed if any associations exist between self-reported dietary intake of polyunsaturated fatty acids and obesity in 7-12 year old US American children from a racially diverse background.*
- *Associations were identified between higher total PUFA intake and lower body fat and intra-abdominal adipose tissue.*
- *Total dietary PUFA intake, as well as the ratio of PUFA to saturated fatty acid intake, as reported by the children, was positively correlated with lean mass.*

Obesity in children, or pediatric obesity, has shown a gradually increasing prevalence over the past three decades, predominantly in many Westernized countries. Worldwide, the proportion of overweight and obese children rose by ~47% between 1980 and 2013. In 2013, in developed countries 23.8% of boys and 22.6% of girls have been reported to be overweight or obese. The prevalence of obesity in children in the US is now estimated to be approximately 17%. The staggering number of children that are overweight and obese is a serious public health concern that has reached the agenda of international organizations and governments. Although a slowing down in the increase has been noted only recently in some countries, the high prevalence of childhood obesity is a concern because the large number of overweight children remain overweight during adulthood. Adult obesity is particularly disquieting in the US, with a recent study reporting only four US states having an obesity prevalence below 30%.

Obesity is defined by an excessive accumulation of white adipose tissue. Obesogenic adipose tissue growth is different from the development of fat tissue in normal development; both the formation of new adipocytes that differentiate from adipose precursor cells present in adipose tissue, as well as the size of adipocytes is increased, compared to adipose tissue in lean persons. Whereas storage of energy in the form of fat is a physiological process, dealing with excess energy triggers adaptations in adipose tissue, which can become pathological. Obesity in adults is associated with a markedly increased risk of other diseases such as type 2 diabetes and cardiovascular disorders. Also in children obesity has been associated with different aspects of metabolic syndrome, although a definition of metabolic syndrome in children proves difficult to establish. Furthermore, a high body mass index (BMI), gains in BMI, and obesity during childhood furthermore confer higher risks of obesity-associated comorbidities during adulthood. Therefore, reducing obesity during childhood is considered to be critical in order to lower adverse health risks later in life. It needs to be mentioned that childhood obesity-associated risks are not irreversible, and attaining a normal weight during adulthood is associated with a risk that is comparable to that of adults who have not been obese or overweight during their childhood. Identification of the relevant factors that drive pediatric obesity, especially those influences that can be reversed, is likely to be helpful in addressing this epidemic.

Obesity in children is a serious public health concern because the number of overweight and obese children has risen markedly during the past three decades, and most overweight children will remain overweight in adulthood.

Many factors are considered to play a role in the advance of obesity in youth, such as: increased accessibility to affordable food in general, and refined carbohydrate-containing food specifically, the promotion of sweet preference by the use of sweeteners in calorie-rich food and beverages, the use of visual cues that promote acquisition of calorie-rich food, and increased portion size. These factors are believed to find their main cause in living in an increasingly calorie-replete environment. A major contributor has also been a reduction in exercise and



increase in sedentary behavior as a result of increased use of indoor activities (computer/games) by children. A relatively new idea explaining the obesity epidemic is that body weight is to a major extent under external control, *i.e.* our brain does not exert an inhibitory control over eating under most circumstances, and we engage in appetitive behavior unless the financial or physical cost of access to food is high. As a result of increased access to food in general, eating and body weight will increase by default. A further line of thinking is that a decreased hippocampal-mediated feedback on energy signals, caused by a Westernized diet with too high intake of saturated fatty acids and total energy, and a lack of specific beneficial nutrients that support hippocampal regulation of food intake and metabolism, may underlie a cognitive defect that allows overconsumption and the development of the overweight state.

The hippocampus exerts an important controlling function in the neurocognitive control over food intake, in particular in the learned control over eating behavior. Habitual omega-3 dietary intake and higher DHA levels in primary school-age children have been linked to higher hippocampal relational memory, whereas saturated fatty acid and sugar intake is negatively related to hippocampal function. Nutrient quality may be of crucial importance to maintain normal neurophysiological control over food choices, intake level and proper metabolic responses to energy intake, including post-ingestive inhibitory influence that limits further food intake. In other words, disturbances in the normal neurophysiological relationship between taste and energy content could contribute to increased food and energy intake in the context of a poor and energy-replete diet, allowing passive overconsumption and leading to overweight.

Specific but poorly defined technological and economic developments over the past decades that affect all socio-demographic groups worldwide have also been suggested to affect people globally and drive the obesity epidemic. Changes in the accessibility of specific dietary nutrients have occurred in parallel with a significantly increased dietary use of vegetable seed oils responsible for a substantially higher intake of linoleic acid. An essential relation between the dietary intake of specific PUFA and risk for developing obesity has been postulated. Exploratory studies in children have found associations between obesity, disturbances in energy metabolism, and low blood levels of EPA/DHA. Some studies have provided indications that in obese children omega-3 LCPUFA levels in blood may be lower than in lean children, but not all studies are supportive. Currently, no conclusive evidence is available that support an unambiguous link between tissue levels of EPA/DHA and alterations in blood lipid profile, insulin sensitivity, and blood pressure in obese children.

In order to establish if any relationship between PUFA intake and obesity in children exists at all, Cardel and colleagues have determined the associations between self-reported fatty acid intake and several indices of adiposity in children age 7-12 years. The study was performed at the Department of Pediatrics and the Anschutz Health and Wellness Center of the School of Medicine at the University of Colorado Denver in Aurora, CO, and the Department of Nutrition Sciences and the Nutrition Obesity Research Center at the University of Alabama, Birmingham, AL, USA. Furthermore, the associations between the ratio of PUFA to saturated fatty acid intakes were assessed in order to determine if higher saturated fatty acid intake in combination with lower PUFA intake might be associated with higher adiposity. Body composition and dietary intake were determined in 311 children with a racially-diverse background (European American, African American and Hispanic American backgrounds, 37, 34 and 27%, respectively). The racial diversity of the study sample is an important aspect since previous studies in this area had focused on study groups with limited population diversity.

Of the children, nearly half were girls (47%), and none had any medical diagnosis or received medication. The mean age of the children was 9.6 years and they were in a peri-pubertal stage. All children were weighed, their height measured, and their fat mass, lean mass, and percentage body fat measured by dual-energy X-ray absorptiometry. Abdominal adiposity was measured by computed tomography scanning (in two-thirds of the children), permitting quantification of intra-abdominal adipose tissue, sub-cutaneous abdominal adipose tissue, and total abdominal adipose tissue. Over half of the children were of normal weight, 23% were overweight and 10% obese. The children were asked to report their dietary intake of different food items by means of two 24-hour recalls taken at two study visits in a one-month period (in the presence of one of their parents). Among the various calculations made from the recall measurements were macronutrient composition, total PUFAs, saturated fatty acids, omega-3 PUFAs, omega-6 PUFAs, and total energy intake. Dietary intake of nutrients was corrected for daily energy intake. The researchers acknowledge the

In this study various aspects of adiposity and its relation to energy-adjusted fatty acid intake were determined in a racially-diverse group of school-age children.

limitations associated with a dietary recall approach to calculating energy intake. Of interest, resting energy expenditure and daily physical activity of all children were also determined. Resting energy expenditure represents most of the daily energy expenditure of children. Socioeconomic status, pubertal status, and genetic admixture were determined for each child as additional covariates.

In order to assess if PUFA intake had any relationship to demographic or specific dietary variables, the researchers first carried out a comparison of the children with a total daily PUFA intake above the mean (13.5 ± 6.7 g/d) with those below the mean. Children with higher total PUFA intake (17.2 ± 7.2 g/d) had significantly lower carbohydrate intake, higher total fat intake, and higher PUFA intake as a percentage of energy. They also ingested significantly higher amounts of omega-3 LCPUFA, alpha-linolenic acid, EPA, DHA, linoleic acid, and arachidonic acid. The ratio of total PUFA to saturated fatty acid intake was nearly double compared to children with a total daily PUFA intake below the average. Importantly, the average daily energy intake, resting energy expenditure, or daily physical activity, was not different between the children with lower or higher total PUFA intake.

Associations between individual dietary variables and measures of body composition and adiposity were determined by multivariate linear regression analysis. Total PUFA intake was found to be positively associated with lean body mass, and negatively associated with the percentage of body fat and intra-abdominal tissue. A higher ratio of PUFA to saturated fatty acid intake was associated with higher lean mass, lower percentage body fat, and lower intra-abdominal fat. Higher intake of both omega-3 LCPUFA and omega-6 LCPUFA was associated with higher lean mass, but not with any measures of adiposity. The ratio of omega-6 to omega-3 PUFA intake was found to be negatively associated with intra-abdominal adiposity, but not with other measures of body composition. All analyses were adjusted for a number of potential confounders such as pubertal stage, sex, socioeconomic status, genetic admixture, and total energy intake (and for height in the case of lean mass).

The results of this study suggest that in a racially diverse group of US American school-age children, a higher self-reported intake of PUFAs and a higher ratio of PUFAs to saturated fatty acids is positively associated with lean mass, and negatively associated with visceral adiposity and the percentage of body fat. It needs to be noted that the results of this study are obtained on a background daily intake of EPA plus DHA that is very low; even in the children with total PUFA intake above the mean, less than 20% met recommended intake levels. In this context, both omega-6 and omega-3 LCPUFA intake was associated with increased lean mass, but not with any measure of adiposity. Perhaps unexpectedly, the ratio of omega-6 to omega-3 intake was found to be negatively associated with intra-abdominal fat mass.

Several research groups have found that omega-6 PUFA and omega-3 PUFA play a role in determining whether adipose tissue displays a metabolically healthy phenotype or an inflammatory phenotype that may drive aspects of the metabolic syndrome. A higher omega-6 to omega-3 PUFA ratio in the Western diet has been implicated as causing greater fat mass accumulation and thus contributes to increased pediatric obesity prevalence. In adult women who were initially of normal weight, the omega-6 PUFA level in red blood cells has been positively associated with developing overweight over a 10-year period. In contrast, adults receiving a diet rich in omega-6 PUFA displayed lower hepatic fat accumulation. The authors of the present study suggest that the role of omega-6 PUFA has not been sufficiently well studied with respect to childhood obesity, and that much of what we know about the role of individual fatty acids in obesity is derived from mechanistic studies carried out on rodents.

As recognized by the authors in this report, the measurement of energy intake from self-reported estimates is problematic. The individual variation in actual energy intake from that calculated from self-reported dietary intake is very large, and it has been argued that dietary energy intake cannot be reliably determined at all from recall measurements. Whether energy intake from self-reported 24-h recall may be less variable in children than in adults is not known. Although not statistically significant, the high PUFA group did exercise more (in minutes). However, no adjustment in the analysis was made for moderate and vigorous physical activity. In order to gather additional support for the validity of the results of this study, replication is needed, as well as the use of more reliable methods for measuring energy intake. The results of this study reveal statistically significant associations, but no further

This study suggests that higher dietary intake of PUFA may be related to leanness and abdominal obesity in school-age children. The results of the study require replication and new controlled intervention studies to establish which specific PUFA species are useful in counteracting the development of the overweight state.

analyses were carried out to minimize random sampling errors (significance levels were not adjusted for multiple comparisons to counteract the increasing error rates associated with multiple comparisons). As indicated by the authors, the results are exploratory, and additional research studies are required.

The important message from this study is that higher PUFA intake, and the ratio of PUFA to saturated fatty acid intake, may be characteristic of children that are less obese and leaner. The strengths of this study are the use of advanced techniques for measuring body composition and adiposity, instead of only BMI, as well as the correction for a number of confounding factors that have not been measured in previous studies, such as ethnic background and energy expenditure. Further well-designed intervention studies are now needed to show a causal relationship between loss of leanness due to low dietary intake of PUFA and/or excess of saturated fatty acid intake in school-age children. The results of this study lend support to the idea that dietary deficiencies and excesses of specific fatty acids may be related to development of adiposity in children. The age group studied here follows a critical age period (5-7 years) in which obesity as part of chronic disease programming becomes more evident. This study represents one further step in assessing if the quality of a child's diet constitutes an important factor contributing to pediatric obesity, particularly in line with the potential to address overweight and obesity by regaining control over food-related cognitive processing.

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No Change in Upper Gastrointestinal Complications after Low-Dose Aspirin with Concurrent Prescription of Omega-3 Fatty Acid Intake

THIS ARTICLE AT A GLANCE

• *This study assessed if in a large group of patients taking low-dose aspirin for thromboprophylactic purposes during their otherwise daily life, the concurrent use of prescription omega-3 fatty acids would affect the incidence of upper-gastrointestinal complications.*

• *Drawing from a large patient register, the authors determined that concurrent use of low-dose aspirin together with prescription omega-3 fatty acids did not change the risk of gastro-intestinal complications.*

Aspirin was the first synthetic pharmacological entity to be brought to the market, more than one hundred years ago. It is still one of the most widely taken drugs in the world, largely due to its useful pharmacological actions in controlling pro-inflammatory and pro-thrombotic activity in acute and chronic inflammatory disorders. In addition to its short-term therapeutic use, primarily as an analgesic and as an anti-platelet agent, long-term prophylactic use of aspirin is now also well established. At minimal effective doses in the range of 50 to 180 mg a day, aspirin can significantly reduce the risk of stroke and myocardial infarction in people with a range of vascular disorders. In particular, the so called "low-dose" aspirin intake, *i.e.* daily oral dosing up to 100 mg, is prescribed by physicians globally for the secondary prevention of heart infarct and stroke. Also, primary prevention with low-dose aspirin is becoming an increasingly attractive prophylactic approach, as a reduced risk of non-fatal myocardial infarction and some types of cancer may outweigh the risk of side effects in specific population and age groups.

The antithrombotic effect of low-dose aspirin is due to a selective inhibition of platelet cyclooxygenase-1 (COX-1) enzyme, responsible for thromboxane A_2 (TXA_2) and prostaglandin formation by platelets. Aspirin irreversibly inhibits COX-1 in circulating platelets thereby reducing TXA_2 formation, significantly reducing the aggregating and thrombogenic potential of platelets. Even at low doses, aspirin also reduces prostaglandin I_2 (PGI_2) formation by arterial endothelial cells, which plays a central role in maintaining the vasculature as a non-thrombogenic surface. However, in contrast to platelets, which have only a limited and variable

capacity to produce new COX-1 enzyme, rapid cyclooxygenase transcription and synthesis (within hours) in vascular beds that produce PGI_2 under basal conditions allows for a net TXA_2 suppression relative to PGI_2 formation. Selective antiplatelet therapy works if aspirin is given at sufficient time intervals, and is of particular relevance in the context of atherosclerosis to avoid thrombosis at unstable plaque surfaces.

Aspirin has a range of additional targets, through the acetylation of several important regulatory enzymes and transcription factors, leading to activation of anti-inflammatory, inflammation resolving, and chemopreventive/antioxidant pathways. Protection of endothelial cell function, lowering of coagulopathies and vascular occlusion, analgesia, direct antitumor effects, and limiting platelet-assisted tumor metastasis are important indications for today's use of aspirin. Short-term aspirin treatment in acute myocardial infarction also has survival benefits that persist long term and are additional to those of fibrinolytic therapy. The favorable thromboprophylactic effect of low-dose aspirin can be increased by prolonged treatment for some years in people with a high risk of occlusive cardiovascular disease. The long-term use of low-dose aspirin is now considered effective in the prevention and treatment of both arterial and venous thrombosis. The permanent inactivation of COX-1 in platelets is central to the wide-ranging pharmacodynamics of aspirin, through changes in platelet physiology affecting multiple organ systems.

A well-known limitation/risk of the use of aspirin is irritation and damage to the mucosa of the stomach and upper intestine. This side effect may involve dyspepsia, abdominal distress and serious tissue lesions involving bleeding, perforations and peptic ulcers, and occurs even at low aspirin doses. The incidence of upper gastrointestinal complications (UGIC) depends to a large extent on a patient's age and un-

derlying individual risk factors. A recent estimate indicates that 29 more cases of major gastro-intestinal bleeding will occur for every 100,000 person-years of primary prevention in healthy persons taking low-dose aspirin. However, in elderly people with existing gastric ulcers, aspirin use can double UGIC rates to over 100 events per 1000 person-years. Concurrent use of other non-steroidal anti-inflammatory drugs (NSAIDs) and anti-coagulant drugs can further aggravate the incidence and severity of gastroduodenal damage. Since concurrent NSAID intake is frequent in patients with chronic inflammatory disorders, a careful evaluation of the benefits of taking aspirin and risks of adverse effects needs to be made on an individual basis.

Among drugs taken concurrently with low-dose aspirin, prescription omega-3 long-chain polyunsaturated fatty acid (LCPUFA), principally EPA and DHA, take up a notable position since indications for omega-3 LCPUFA intake overlap to a certain extent with the indicated use of aspirin. EPA and EPA/DHA combinations are prescribed for the secondary prevention of cerebro- and cardiovascular diseases, primarily to achieve a reduction in elevated plasma triglyceride levels. Although even relatively low doses of omega-3 LCPUFA have measurable effects on *ex vivo* platelet aggregation, they do not induce bleeding in a clinically significant manner at normal prescribed doses, alone or in combination with other drugs such as aspirin. Since omega-3 LCPUFA enhance the anti-platelet and thrombotic activity of aspirin, reservations on concurrent intake of aspirin and omega-3 LCPUFA do remain with respect to the possibility of enhanced risks for gastro-intestinal tract damage and bleeding. Preclinical research suggests that omega-3 LCPUFA also exert tissue protective effects in the stomach, but it is not yet clearly established if omega-3 PUFA intake modifies the side effect profile of low-dose aspirin intake in humans. Although the combined intake of omega-3 fatty acids and low-dose aspirin is deemed to be safe from results in controlled clinical trials, no information is available about the safety of omega-3 LCPUFA and low-dose aspirin intake from observational studies.

A real-life problem of the use of low-dose aspirin for thrombo- prophylaxis is the risk of upper gastrointestinal tract injury. This side effect is of significant relevance since aspirin use is extremely common.

In order to evaluate if any relationship exists between the concurrent exposure to low-dose aspirin and omega-3 fatty acids, and upper gastrointestinal complications (UGIC) incidence, a large case-controlled analysis was recently carried out in Italian patients. This study was performed by Roberto and colleagues from the Epidemiology Unit at the Regional Agency for Healthcare Services of Tuscany, and the Italian College of General Practitioners and Primary care, both in Firenze, Italy. The estimation of UGIC incidence in patients concurrently exposed to low-dose aspirin and medications containing omega-3 fatty acids was achieved through analysis of patient data stored in electronic medical records contained in a large Italian general practice database, the Health Search-IMS Health Longitudinal Patients Database. This primary care database is maintained by the Società Italiana de Medicine Generale e delle Cure Primarie, and contains clinical data from over 1.5 million patients. Records suitable for research purposes are provided by a network of 700 primary care physicians across Italy. The researchers extracted the study cohort and their associated clinical information, drug prescriptions, and lifestyle information based on several criteria: patients were at least 18 years old and had been diagnosed with cerebrovascular ischaemic or coronary heart disease during a 10-year period (from 2002 until end 2012). Patients that already had a gastrointestinal disease or were already prescribed aspirin or omega-3 fatty acids prior to the defined study period, or for whom less than one year of clinical data was available, were excluded.

Of the 20,287 patients who suffered a stroke or heart infarct during the 10-year period, 1,976 individuals had also experienced an UGIC. These cases were identified using a validated extraction strategy using standard diagnostic ICD-9-CM codes, as well as keywords used in the free text provided by physicians in medical reports. The specific codes and keywords employed identified diagnoses of ulcers, bleeding, perforations, and inflammatory events in the oesophagus, stomach, jejunum and duodenum. The date of first occurrence of an UGIC was taken as an index date, and used to compare UGIC incidence with control patients. An UGIC incidence rate was calculated for the entire study cohort by dividing the number of cases by the summed total number of person-years during follow-up (the time for each patient in

This study assessed if in a large group of patients that had suffered a stroke or heart infarct and were taking low-dose aspirin for thromboprophylactic purposes during their otherwise daily life, the concurrent use of prescription omega-3 fatty acids would affect the incidence of upper-gastrointestinal complications.

the cohort until the end of the study period, the index date in case of UGIC, death, end of registration with their physician, diagnosis of cancer, or diagnoses of a different gastro-intestinal disease). Up to ten control patients that had not suffered an UGIC during the study period were randomly selected from the remaining study cohort, and matched to each UGIC case based on index date, date of cohort entry, age, gender, and duration of follow-up. It was possible to match 1908 UGIC cases up to ten controls each.

Patients in the study cohort (average age 70 years, 57% men) had an overall UGIC incidence rate of 22.8 per 10,000 person-years. Comorbid disease incidence was overall similar between cases and matched controls, except a higher percentage of cases had respiratory disease (chronic obstructive airway disease and asthma), and reduced incidence of diabetes and ischaemic stroke. Use of prescription low-dose aspirin was identified from the ATC therapeutic drug codes B01AC06 and B01AC56, and prescription omega-3 fatty acids with C10AX06 (omega-3-triglycerides including other esters and acids). Concurrent pharmacotherapy with other drugs, measured in the year preceding the index date, was significantly higher in cases than controls, in particular the use of proton-pump inhibitors (74% vs 28%). Follow-up was 2.5 years on average for cases and controls.

To determine if any relationship between concurrent intake of low-dose aspirin and omega-3 fatty acids and UGIC might exist, the researchers took three approaches: a primary analysis that addressed the "recency" of concurrent intake and UGIC incidence, a secondary analysis that investigated the effect of duration of concurrent intake in current users (less than 30 days before UGIC), and a sensitivity analysis that determined how different values of the independent variable (timing and duration of concurrent intake) impacted UGIC incidence.

For the primary analysis, three exposure periods of concurrent low-dose aspirin and omega-3 fatty acid use were defined; *i)* current use (from 30 days prior up to the index date where UGIC was diagnosed; 1.7% of cases), *ii)* recent use (from 31 to 60 days preceding the index date; 2.4% of cases), and *iii)* past use (from 61 days to one year preceding the index date; 1.3% of cases). A reference group of non-users included the cases in which no concurrent use of prescription low-dose aspirin and omega-3 fatty acids was present. The number of days of concurrent use in cases and matched controls in each of the three analyzed exposure periods was similar.

The results of the primary analysis showed that no significant association could be identified between UGIC incidence and the recency of concurrent use of prescription omega-3 fatty acids and low-dose aspirin. Odds ratios (incidence rate ratios) for UGIC were indistinguishable between current, recent or past concurrent users, and when compared to non-users that did not have concurrent use. Corrections made for a range of covariates that could be expected to affect the relationship between UGIC incidence and low-aspirin plus omega-3 fatty acid intake did not reveal any statistically significant differences in the observed odds ratios.

In the secondary analysis, the associations between the duration of concurrent low-dose aspirin and omega-3 fatty acids exposure, and UGIC were determined for cases that had been taking both prescription drugs within a 30-day period prior to UGIC (*i.e.* current use). Assessment of associations between UGIC incidence and the tertiles of duration of concurrent use showed that concurrent use up to 9.66 days during the month prior to UGIC was protective with an odds ratio of 0.42 after adjustment for a range of covariates. Longer periods of concurrent use in the month prior to UGIC occurrence did not display statistically significant associations with the odds of experiencing an UGIC.

A subsequent sensitivity analysis did not support the finding that the short-term recent concurrent use was protective for UGIC. When a lag time was applied to model if exposure periods that preceded the index date by 30, 60, or 90 days affected the UGIC incidence, thereby excluding any potential for reverse causation (*i.e.* people halting their aspirin and omega-3 intake because they suffered prodromal effects leading up to UGIC, such as noting a bloody stool or abdominal pain), no difference in UGIC incidence was found for current use. Statistically significant increases in UGIC inci-

This study suggests that concurrent intake of low-dose aspirin and omega-3 fatty acids in patients at risk of recurrent atherothrombotic vascular complications does not heighten the risk for upper gastro-intestinal lesions compared to patients not taking low-dose aspirin and prescription omega-3 fatty acids together.

dence were observed when modeling concurrent use at the later shifted periods. The authors conclude that the observed early "protective" effect is likely due to chance. Overall, no convincing effect of omega-3 fatty acid intake on low-dose aspirin-induced UGIC incidence could be found. An additional comparison of UGIC risk in people with concurrent use with those patients only receiving low-dose aspirin monotherapy also did not reveal any significant effect.

The results of this study show that in elderly Italian patients taking low-dose aspirin for thromboprophylactic purposes during their otherwise normal daily life, the concurrent use of prescription omega-3 fatty acids does not aggravate the upper gastrointestinal complications that accompany the use of aspirin. This is an important and significant observation given the widespread use of aspirin worldwide. The findings reported here may instruct new studies that document the pharmacological efficacy of concurrent aspirin and omega-3 LCPUFA intake, armed with the knowledge that taking them together likely does not make the side effects of low-dose aspirin on the upper gastro-intestinal tract worse.

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GUEST ARTICLE

On the Contribution of Plasma Lysophospholipid and Non-Esterified Docosahexaenoic Acid to the Brain

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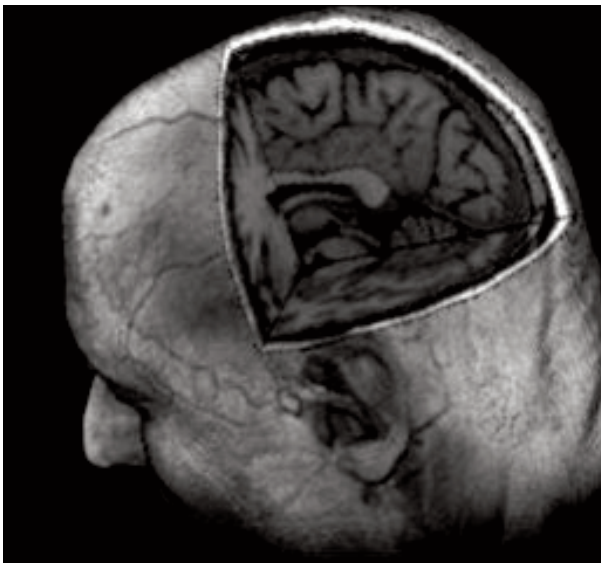


The brain has a lot of docosahexaenoic acid

The brain is particularly enriched with the polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA; 22:6n-3) while being almost devoid of other omega-3 PUFA, such as eicosapentaenoic acid (EPA). Within the brain DHA makes up around 10% of the total fatty acids and about 50% of the PUFA, corresponding to a concentration of about 10,000 nmol/g or about 5 grams in the whole adult brain[1]. DHA is especially enriched in the grey matter and the synapses where it participates in signal transduction, either directly or upon bioconversion to a series of lipid mediators including protectin D1 and docosahexaenoyl ethanolamide. Collectively, DHA and its metabolites have been shown to regulate cell survival, cell growth and neuroinflammation within the brain[2-4]. Because the brain cannot synthesize DHA de novo, the brain relies on the uptake of DHA from the blood to replace the DHA consumed in metabolic reactions.

Lipoproteins

The plasma pools and mechanisms by which fatty acids such as DHA enter the brain have long been debated[5-7]. Plasma pools thought to supply the brain with DHA included lipoproteins, especially low-density lipoproteins (LDL), lyso-phospholipids containing DHA and non-esterified DHA. In order to test the hypothesis that the LDL receptor was necessary for maintaining brain DHA levels, we measured DHA concentrations in wild type and LDL receptor knock-out mice[8]. However, a bit to our surprise, there was no difference in brain DHA concentrations between knock-out and wild type controls. This does not mean that the LDL receptor is not involved in maintaining brain DHA levels or in the transport of DHA, but rather that it is not necessary and that other mechanisms are sufficient. Interestingly, VLDL receptor knock-out mice also did not have lower brain DHA levels than wild type controls[9]. While using knock-outs can provide useful information, even if we lowered DHA in the absence of a candidate transporter, it would not have proved that the candidate was directly important for transport or uptake, nor would the quantitative relevance have been known.

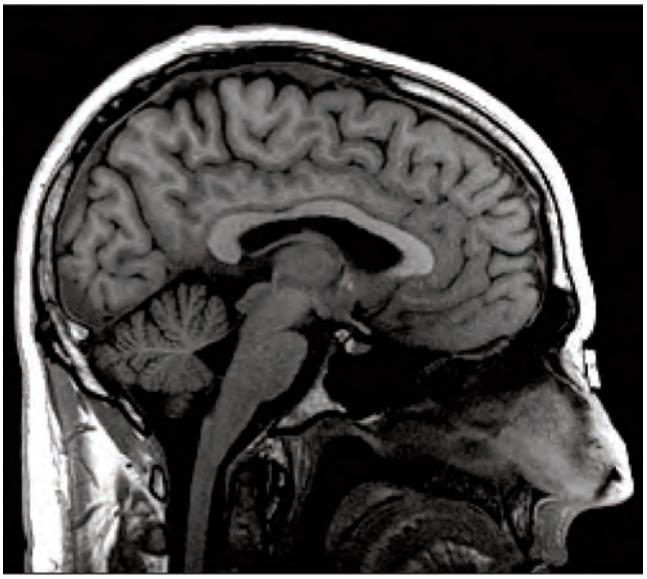
Non-esterified docosahexaenoic acid

Stanley Rapoport's laboratory developed a method to quantitate the rate of uptake of plasma non-esterified fatty acids into the brain[10]. The method involves infusing a labelled fatty acid tracer intravenously into the unanaesthetized rodent for 5 minutes upon which the label is measured in the brain. The short duration is important for several reasons, including that if the infusion continues for too long, non-esterified fatty acids, including DHA, are taken up by the liver and secreted into the lipoprotein pool esterified to phospholipids and other glycerides as well as in cholesteryl esters. Thus, upon detecting the tracer fatty acid in the brain, it would be unclear which plasma pool(s) were contributors to brain uptake; however, this problem is avoided with the acute infusion model. During the infusion blood is taken and the amount of the tracer in the brain is corrected for the area under the curve of the infused tracer giving a unidirectional incorporation coefficient. By multiplying the incorporation coefficient by the plasma concentration of the non-esterified fatty acid, the uptake rate into the brain or brain phospholipids can be determined. This method has been used by several labs to determine the rate of uptake of DHA, arachidonic acid, EPA, erucic acid, linolenic acid, alpha-linolenic acid, docosapentaenoic acid and palmitic acid into the brain[11-16]. While this approach is quantitative, it was necessary to figure out if the amount entering the brain from the plasma non-esterified DHA pool was a major or minor contributor to the brain. One piece of evidence that the non-esterified DHA pool was a major contributor to brain DHA was that the rate of DHA entry into the brain in control animals overlapped with the rate of brain consumption of DHA in animals that

were either on a diet deprived of or containing n-3 PUFA[17]. Because the rate of DHA entering the brain was approximating the rate of DHA consumption, and brain DHA levels are constant in adult rodents, this provided evidence that the non-esterified pool is a major contributor to brain DHA uptake[18]. Furthermore, consistent with this observation, while the brain can elongate some alpha-linolenic acid to DHA, the rate of the reaction was quantified *in vivo* and found to be just a very small fraction of the DHA uptake rate from the non-esterified pool[14], a finding that was later also demonstrated with the conversion of EPA to DHA[15].

Lyso-phosphatidylcholine

Lyso-phosphatidylcholine (LPC) containing DHA can be present in the "free" plasma pool or as part of lipoproteins. Earlier work in squirrel monkeys had suggested that plasma LPC esterified to palmitic acid was an important source of both phosphatidylcholine fatty acid and choline for the brain[19]. This work was followed up and significantly advanced by Michel Lagarde's group who demonstrated that several LPCs (palmitic, oleic, linoleic and arachidonic acids) not only entered the brain, but upon an acute intravenous perfusion of labeled LPC, more labelled fatty acid was present in the brain as compared to administration of the non-esterified tracer[20]. Interestingly, their work also appeared to demonstrate that arachidonic acid esterified to LPC had a longer



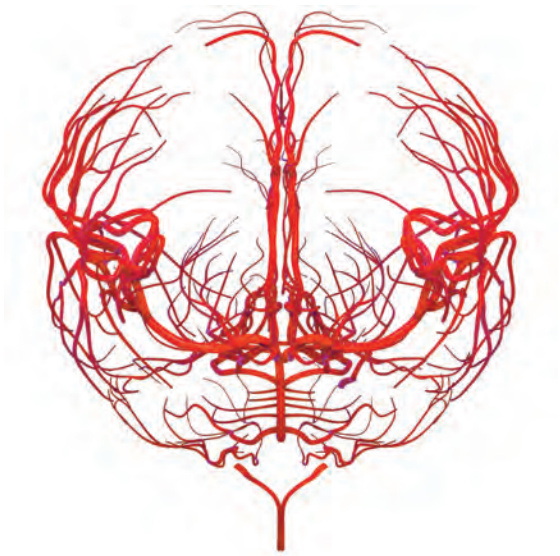
plasma half-life than non-esterified arachidonic acid. This work was followed up by demonstrating that upon an intravenous perfusion, more labelled DHA was present in the brain when the tracer was given as LPC-DHA as compared to non-esterified DHA[21]. Furthermore, this paper demonstrated (although not tested statistically) that several LPCs, including LPC-DHA, had longer plasma half-lives than non-esterified DHA. While this earlier work might have seemed to be at odds with the observation that non-esterified DHA appeared to be entering the brain at the same rate by which DHA was being consumed in the brain, there were several important differences in the studies. Firstly, the work on LPC-DHA was mostly performed in young (20-day old) rats, which might be important given that the young brain has the additional burden of accreting DHA. Thus, it is possible that additional mechanisms of uptake are present in the young rat as compared to the adult. Also, the terms "preferential" or "more efficiency" were used to describe the uptake results[20-22], which was reasonable given that more labelled DHA was present in the brain upon intravenous LPC-DHA administration compared to non-esterified DHA.

The next papers

Building, in part, on the work of Lagarde and colleagues, David Silver's laboratory performed a series of elegant experiments in wild type and Mfsd2a knock-out mice[23]. Mfsd2a knock-out mice had about 8 percent DHA in brain phospholipids, while wild type mice had close to 20 percent. This and a series of other observations led them to explore the relationship between Mfsd2a and LPCs, including LPC-DHA as well as non-esterified DHA. While indirect genomic and biochemical evidence was considered supportive of an important role for LPC-DHA and the brain, the only direct evidence related to uptake presented were studies examining the appearance of labelled DHA upon non-esterified DHA and LPC-DHA injection. In these experiments, which were broadly similar to earlier studies, tracers of LPC-DHA and non-esterified DHA were perfused intravenously and the appearance of the label in the brain was measured two hours later. Again, similar to the previous work, the amount of labelled DHA in the brain after two hours was higher upon intravenous LPC-DHA administration than non-esterified DHA administration. However, surprisingly (at least to us) this paper concluded that LPC-DHA was the major source of DHA supplying the brain. It was not clear if the tracer experiments and/or the indirect genomic and biochemical data were the foundation of this conclusion. Nevertheless, and ignoring that within about 15 minutes, let alone two hours after intravenous administration of the tracers, they would be present in multiple plasma pools and it would be impossible to determine which pool had contributed DHA to the brain, there were no experiments comparing the rate of entry of the two candidate pools of DHA into the brain. This seemed especially important given the longer plasma half-life of LPC-DHA (several minutes) compared to unesterified DHA (about 30 seconds). If a tracer has a longer half-life, its entry into the brain could be slower, but given the length of the experiment it would have more opportunity to enter the brain. For instance, in about 2.5 minutes, the non-esterified DHA tracer would be virtually cleared from

the plasma while there would still be about 50% of the dose of the LPC-tracer circulating and able to enter brain. Furthermore, one has to remember that while the tracer levels are not at steady-state, the unlabeled (tracee) pools would be at steady-state and not approaching zero.

In order to shed light on this controversy we conducted a series of kinetic experiments[24]. First, we replicated the finding that plasma non-esterified DHA entered the brain at a rate approximating DHA being consumed in the brain. Second, we gave labelled DHA orally and observed its appearance in many plasma pools, including the LPC-DHA pool and the brain. In this experiment we were able to demonstrate that applying the experimentally derived uptake coefficient for non-



esterified DHA to the oral experiment we could explain most, if not all, of the label in the brain. Collectively, the results of these experiments left little room for the uptake of other plasma DHA pools into the brain. We then attempted to replicate the study from the Silver's group. Two hours upon intravenous administration of labelled LPC or non-esterified DHA, there was more labelled DHA in the brain of animals that received LPC-DHA. However, we also measured other plasma DHA pools during the study. As we expected, labelled DHA, under both conditions, appeared in numerous plasma pools during the two hours, making it impossible to tell which pool(s) had contributed DHA to the brain. Furthermore, we calculated that the amount of labelled LPC-DHA in the plasma that the brain is exposed to was about five times more than non-esterified DHA during the course of the experiment, entirely consistent with the longer plasma half-life of LPC-DHA. When we for the exposure to the brain modelled the net rate of uptake, it was evident that the rate of non-esterified DHA into the brain was many times higher than LPC-DHA. In our last experiment, in part to avoid the issues with the longer experiments, we continuously infused labelled LPC-DHA or non-esterified DHA for five minutes and applied the modelling developed by Stanley Rapoport. Again, consistent with the longer half-life of LPC-DHA than non-esterified DHA, its contribution to plasma radioactivity was higher and it continued to rise during the study (because it is not being cleared as fast). Finally, we found that the plasma non-esterified pool was supplying about 10 times more DHA to the brain than LPC-DHA.

But LPC-DHA is still very important

Our findings that non-esterified DHA is the major plasma pool supplying the brain do not negate the importance of the LPC-DHA pool. Firstly, as has been known for over 50 years LPCs are important sources of choline to the brain. Secondly, even though LPC-DHA is not the major source of brain DHA under normal physiological conditions, it is still a viable therapeutic approach to target the brain with DHA. While it may not seem intuitive, a higher percentage of exogenously administered (i.e. a bolus intravenous dose) LPC-DHA will reach the brain as compared to non-esterified DHA over time. This is because the administered LPC-DHA will circulate in the plasma and continue to be taken up by the brain long after the administered non-esterified DHA has been taken up (note: *in vivo* the plasma levels are maintaining at a steady-state). Thus, the use of LPC-DHA is a reasonable approach for targeting the brain. In fact, much success has been ascribed to LPC-DHA, relative to non-esterified DHA, in preclinical models of stroke[25]. Studies are currently making more stable derivatives of LPC-DHA which may enhance its suitability for delivery to the brain[26,27] and an obvious area of research would be to try and target the plasma LPC-DHA pool via oral delivery. It will also be important to examine these mechanisms during times of increased brain DHA demand (i.e. brain growth) to test the relative contribution of plasma pools. There may very well be multiple mechanisms that are upregulated to supply the brain with DHA during early brain development. However, because the uptake of non-esterified DHA has been estimated in adult humans using positron emission tomography to be about 2.4-3.8 mg per day[28,29], we can now look at the partitioning of DHA between the brain and tissues as well as the role of diet, disease and other physiological conditions in maintaining this contribution to the brain[30].

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